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MALDI imaging MS analysis of drug distribution in tissue: the right time!(!?)

“The application of MALDI-IMS to determine the tissue distribution of drugs and their metabolites is the type of innovative technology that can have a dramatic impact on both drug discovery and development.”

Keywords: MALDI-imaging MS ■ molecular histology ■ tissue distribution

The goal of pharmaceutical companies is to develop and market safe and efficacious drugs for unmet medical needs. Continuous improvements in technology and the accumulation of scientific knowledge should facilitate more effective means to achieve this goal. Unfortunately, there has been a significant decline in the number of new medicines approved, and at the same time the cost of research and development has soared [1]. Consequently, there is greater scrutiny associated with the return on R&D investment. It is precisely because of these challenges for the pharmaceutical industry that the opportunities and necessity for scientific innovation have never been greater. Many fundamental aspects of drug discovery and development remain inadequately addressed, including knowing the location of the drug and its metabolites in tissue. The emerging technology of MALDI imaging MS (IMS) has the potential to be a ‘game changing’ technique in determining tissue distributions [2–5]. The realization of its potential will depend on how this technology is implemented into the discovery and development environments.

The importance of drug distribution

Understanding the tissue distribution of drugs and their metabolites is at the core of drug discovery and development, impacting pharmacology, safety, pharmacokinetics (PK), metabolism, transport and drug–drug interactions (DDIs). However, much of our *in vivo* understanding of pharmacodynamics (PD) and PK in tissue is derived from surrogates such as blood/plasma or excreta. While there are practical advantages of blood/plasma analyses, it is also becoming clear how drug and metabolite tissue concentrations and distributions are not well represented by these types of analyses. Historically, we have

relied on autoradiography to provide quantitative tissue distribution of drug-related material, or more precisely the distribution of the radiolabel. Autoradiography provides a composite image of all components containing the radiolabel, but provides no structural information. Metabolites not containing the radiolabel are not represented. Even coupling this method with LC–MS/MS analysis of the tissue homogenate does not provide the distinct distributions of the drug and its metabolites. Therefore, even this combined analytical approach is very limited in addressing undesirable safety or pharmacology issues associated with drug dosing.

A safety finding in preclinical or clinical studies necessitates a project team to answer a series of questions that begin with: “Is the finding related to the drug or a metabolite?” Risk mitigation and the future of the compound or program depend on an accurate assessment of molecular species responsible and a mechanistic hypothesis. A similar situation occurs when unexpected secondary pharmacology is observed. Again, the starting point of risk assessment is the identification of the molecular perpetrator in the target tissue. Blood/plasma analysis and autoradiography simply cannot adequately address this type of risk assessment.

MALDI-IMS, being an MS-based technique, does provide the structural characterization for drug-related material in tissue. And because no labeling is involved, all metabolites and their corresponding distributions within the tissue can be mapped. By combining histology images with the molecular images from MALDI-IMS, an imaging modality that integrates molecular structure and biological morphology is obtained. This ‘molecular histology’ can serve as a common imaging platform for pathologists, toxicologists, physicians, biologist and chemists.

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Implementation

There are many opportunities to apply MALDI-IMS in drug discovery and development. In the discovery setting, applications might focus on differentiating a few molecules based on target engagement. These experiments may include MALDI-IMS, in a time-course study, with an appropriate animal model to give more explicit information on PD/PK relationships for the potential drug candidates. Data from such studies might also be used by modeling groups in order to build better kinetic models for the tissue and target of interest. Beyond exogenous drugs, MALDI-IMS captures numerous molecular components in tissues, including endogenous metabolites, lipids, peptides and proteins. In studies where there are changes in tissue concentration of an endogenous species as a result of target pharmacology, MALDI-IMS can capture both drug engagement of the target and a relevant PD change in the same experiment.

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“...MALDI imaging MS has the potential to be a ‘game changing’ technique...”

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MALDI-IMS in development (e.g., post-candidate selection; $\geq$ Phase I) will likely focus on ‘solving problems’ as opposed to ‘avoiding problems’ in discovery. Consequently, studies will generally involve safety issues, tend to be more mechanistic in nature and consist of retrospective as well as prospective studies. For instance, in a drug-induced liver injury (DILI) investigation, an initial MALDI-IMS experiment may focus on correlating the drug and its metabolite distributions with the liver injury. Depending on the outcome of the ‘molecular histology’, hypothesis testing can be achieved in follow-up studies where changes in pathogenesis are altered by study design. MALDI-IMS experiments can be conducted alongside more traditional analyses such as clinical chemistry, gene expression and microscopy. Furthermore, because addressing issues such as DILI requires an extensive collaboration of scientists, MALDI-IMS bridged to histology provides a common platform for physicians, pathologists, toxicologists, biologists, DMPK scientists and chemists. In my opinion, this is the most significant advantage and potential impact of MALDI-IMS in drug discovery and development: it fosters collaborative science.

There do not appear to be any limitations on the types of tissues that can be studied by MALDI-IMS; however, some tissues will be

easier to section and prepare than others. The successful implementation of a MALDI-IMS strategy will be based on clearly defining the desired outcome of the experiment. In instances where a comparison of tissue concentrations for drug candidates suffices, LC-MS/MS methodology could prove to be more efficient. Currently, MALDI-IMS would not be considered a high-throughput methodology and, therefore, resources should be used judiciously. Furthermore, because the acquisition of high spatial resolution images is more technically challenging, consideration should be given to the resolution necessary in the ion images to correlate with the histology. For instance, the brain is well compartmentalized; therefore, brain distribution studies may not require high spatial resolution images to effectively correlate with histology images. In contrast, IMS studies in liver, disparagingly referred to by some as a ‘bag of hepatocytes’, may require more spatial resolution to resolve subcompartments.

Realizing the potential

So what issues stand in the way of MALDI-IMS realizing its potential role as a ‘game changer’ in R&D? Most simply stated: poor implementation of the technology, including the lack of sufficient experimentation and validation of results, will result in the potential of MALDI-IMS not being realized. Despite recent significant advancements in both hardware and software associated with sample preparation, data collection and analysis, MALDI-IMS has not reached the state of a ‘plug and play’ methodology. There is still an ‘art-form’ to generating valuable data, and therefore potential practitioners need to be committed experimentalists.

The sample preparation stage in MALDI-IMS is critical for obtaining reproducible high-quality results. This includes cryosectioning of the frozen tissue and the optimized application of the matrix solution to the tissue. These two skill sets are new to most mass spectrometrists, often requiring support from trained histology staff to assist with both cryosectioning and thaw mounting of tissue on MALDI slides. Unfortunately, at this time the art of matrix application is best achieved through methodical experimentation, review of literature methods, as well as consulting with IMS workshops and networks such as the advanced imaging MS (AIMS) course and workshop at Vanderbilt University (TN, USA) and the European Cooperation in Science and Technology (COST) Initiative in

Mass Spectrometry Imaging. It must be stressed that the MS hardware used to collect the data cannot compensate for poor sample preparation.

“To successfully navigate the complexities of systems biology and pathogenesis, greater collaborative science is required.”

The same analytical advantages and limitations apply to MALDI-IMS as for other MS-based techniques. Specifically, the impact of ionization efficiency and suppression must be addressed with respect to the quantification strategy employed and the ion images observed. Drug and metabolite MALDI-IMS, without an element of quantification, has limited value for decision-making purposes. Fortunately, this is an area that is actively being developed in the scientific community. Literature methods range from more traditional homogenate LC-MS/MS analyses using standards to a variety of methods more closely related to the MALDI-IMS experiment.

In the end, the practitioner of MALDI-IMS needs to have the skill and expertise to confidently acquire high-quality data that accurately reflect the tissue distribution of targeted analytes. Acquiring this experimental expertise, in a work environment driven by tight timelines and rapid turnaround, is demanding. But the return on that investment is significant because it will drive collaborative and innovative science.

The big picture

With the current challenges facing the pharmaceutical industry, there has never been a better time for innovative ideas and technologies. The application of MALDI-IMS to determine the tissue distribution of drugs and their metabolites

is the type of innovative technology that can have a dramatic impact on both drug discovery and development. When integrated with histology images a combined molecular histology imaging modality emerges that integrates molecular structure with biological morphology. This unique imaging combination provides a common platform for physicians, pathologists, biologists and chemists, and therefore facilitates collaborative science among the various disciplines. To successfully navigate the complexities of systems biology and pathogenesis, greater collaborative science is required. Both the potential and applications of MALDI-IMS to determine drug and metabolite tissue distribution are well supported by literature publications. The successful implementation of this technology in its current development requires an ‘early adopter’ strategy. This means that a commitment to learn and optimize through experimentation, as well as the application of sound analytical practices to verify and validate findings, are essential. Time constraints will lead to inconsistent results and a short-lived interest in the methodology. Therefore, we must recognize the strengths and limitations of this technique and design studies that play to its unique attributes. The time is right for those willing to be early adopters to open new scientific frontiers with MALDI-IMS.

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