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Original article

Utility of imaging mass spectrometry (IMS) by matrix-assisted laser desorption ionization (MALDI) on an ion trap mass spectrometer in the analysis of drugs and metabolites in biological tissues

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Abstract

Introduction: The properties and potential liabilities of drug candidate are investigated in detailed ADME assays and in toxicity studies, where findings are placed in context of exposure to dosed drug and metabolites. The complex nature of biological samples may necessitate work-up procedures prior to high performance liquid chromatography-mass spectrometric (HPLC-MS) analysis of endogenous or xenobiotic compounds. This concept can readily be applied to biological fluids such as blood or urine, but in localized samples such as organs and tissues potentially important spatial, thus anatomical, information is lost during sample preparation as the result of homogenization and extraction procedures. However, the localization of test article or spatial identification of metabolites may be critical to the understanding of the mechanism of target-organ toxicity and its relevance to clinical safety. **Methods:** Tissue imaging mass spectrometry (IMS) by matrix-assisted laser desorption ionization (MALDI) and ion trap mass spectrometry (MS) with higher order mass spectrometric scanning functions was utilized for localization of dosed drug or metabolite in tissue. Laser capture microscopy (LCM) was used to obtain related samples from tissue for analyses by standard MALDI-MS and HPLC-MS. **Results:** In a toxicology study, rats were administered with a high dosage of a prodrug for 2 weeks. Birefringent microcrystalline material (10–25 μm) was observed in histopathologic formalin-fixed tissue samples. Direct analysis by IMS provided the identity of material in the microcrystals as circulating active drug while maintaining spatial orientation. Complementary data from visual cross-polarized light microscopy as well as standard MALDI-MS and HPLC-MS experiments on LCM samples validated the qualitative results obtained by IMS. Furthermore, the HPLC-MS analysis on the LCM samples afforded a semi-quantitative assessment of the crystalline material in the tissue samples. **Discussion:** IMS by

Abbreviations: CID, collision-induced dissociation; HPLC, high performance liquid chromatography; IMS, imaging mass spectrometry; LCM, laser capture microdissection; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MW, molecular weight; *m/z*, mass-to-charge; NL, normalized; SRM, selected reaction monitoring.

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MALDI ion trap MS proved sensitive, specific, and highly amenable to the image analysis of traditional small molecule drug candidates directly in tissue.

Keywords: Drug distribution and deposits; Imaging mass spectrometry (IMS); Ion trap mass spectrometer (MS); Laser capture microdissection (LCM); High performance liquid chromatography-mass spectrometry (HPLC-MS); Matrix-assisted laser desorption ionization (MALDI); Qualitative and semi-quantitative analysis; Rat; Spleen tissue

1. Introduction

The understanding of *in vivo* behavior of drug candidates is essential to pharmaceutical development. Compound properties and possible liabilities are investigated in detailed ADMET (absorption, distribution, metabolism, elimination, and toxicology) studies in various animal species (Davis & Riley, 2004; Li, 2002; Lin et al., 2003; Wilson, White, & Mueller, 2003). Findings from toxicity studies have to be placed in context with exposure to test article, i.e., dosed drug, or metabolites, sometimes to the degree of specific localization with a tissue.

The complex nature of biological samples (plasma, bile, urine, tissue) often necessitates sample preparation procedures followed by bioanalytical high performance liquid chromatography-mass spectrometric (HPLC-MS) assessment of endogenous or xenobiotic analytes (Ackermann, Berna, & Murphy, 2002; Jemal, 2000). This presents no issue for fluidic samples but potentially important spatial, thus anatomical information is lost when tissue samples are homogenized and extracted to determine concentration of test article and/or metabolites. In non-clinical toxicology studies the direct and non-invasive identification and quantification of endogenous compounds or drug-related allocation or deposits in animal tissue, while preserving the spatial profile and the tissue architecture, can be a critical piece of *in vivo* biological information in understanding the mechanism of target-organ toxicity and its relevance to clinical safety.

This issue of intact tissue analysis can be addressed by the use of *in vivo* and *ex vivo* imaging techniques (Blankenberg, 2003; Koo, 2005; Stoeckli & Farmer, 2004; Williams, 2006) such as computer tomography, whole body radiography, positron emission tomography, fluorescence microscopy, nanotechnology, infrared imaging, magnetic resonance imaging, and nuclear magnetic resonance, to name a few. However, these methods can be limited by sensitivity, spatial resolution, and the necessary synthesis of reagents or isotopically labeled compounds.

An emerging complementary label-free technology is "imaging mass spectrometry (IMS)" (Atkinson, Prideaux, Bunch, Warburton, & Clench, 2005; Caprioli, Farmer, & Gile, 1997; Chaurand & Caprioli, 2002; Chaurand, Cornett, & Caprioli, 2006; Chaurand, Schwartz et al., 2004; Chaurand, Norris, Cornett, Mobley, & Caprioli, 2006; Chaurand, Schwartz, & Caprioli, 2002; Chaurand, Schwartz, & Caprioli, 2004; Chaurand, Schwartz, Reyzer, & Caprioli, 2005; Maarten Altelaar, van Minnen, Jimenez, Heeren, & Piersma, 2005; Reyzer & Caprioli, 2005b; Rohner, Staab, & Stoeckli, 2005; Rubakhin, Jurchen, Monroe, & Sweedler, 2005; Stoeckli & Farmer, 2004; Stoeckli, Chaurand, Hallahan, & Caprioli, 2001) which affords a sensitive and specific two-dimensional, potentially three-dimensional (Crecelius et al., 2005), ion density map or ion image of drugs (Bunch, Clench, & Richards, 2004; Hsieh et al., 2006; Reyzer, Hsieh, Ng, Korfmacher, & Caprioli, 2003; Troendle, Reddick, & Yost, 1999; Wang, Jackson, McEuen, & Woods, 2005), metabolites (Chen, Hsieh, & Korfmacher, 2006), and endogenous compounds (Chaurand, Stoeckli, & Caprioli, 1999; Hutchinson et al., 2005; Jackson, Wang, & Woods, 2005a, Jackson, Wang, & Woods, 2005b) in tissue samples or in whole body sections (Khatib-Shahidi, Andersson, Herman, Gillespie, & Caprioli, 2006), potentially exploitable for biomarker discovery studies (Maddalo et al., 2005; Reyzer & Caprioli, 2005a) and in diagnostic histopathology (Caldwell, Holt, & Caprioli, 2005; Chaurand, Sanders, Jensen, & Caprioli, 2004). Frozen tissue samples are cut with a cryostat into approximately 10 μm-thick sections, which are mounted onto an object stage. The sample surface is directly scanned using mass spectrometric microprobes such as secondary ion mass spectrometry (Brunelle, Touboul, & Laprevote, 2005; Todd, Schaaff, Chaurand, & Caprioli, 2001), desorption electrospray ionization (Cooks, Ouyang, Takats, & Wiseman, 2006), surface sampling probe electrospray ionization (Van Berkel & Kertesz, 2006), or matrix-assisted laser desorption ionization (MALDI) (Caprioli et al., 1997, Garrett & Yost, 2006; Todd et al., 2001; Troendle et al., 1999). In the case of MALDI IMS, a suitable photoactive MALDI matrix is applied to the tissue surface using methodologies (Schwartz, Reyzer, & Caprioli, 2003) such as immersion, airbrush, electrospray, acoustic deposition (Aerni, Cornett, & Caprioli, 2006), or "printer technique" (Baluya, Garrett, & Yost, 2006; Frappier, Khatib-Shahidi, & Caprioli, 2006; Groseclose, Andersson, & Caprioli, 2006; Patel et al., 2006; Shimma, Furuta, Ichimura, Yoshida, & Setou, 2006). The analytes migrate to the tissue surface and co-crystallize with the MALDI matrix (Crossman, McHugh, Hsieh, Korfmacher, & Chen, 2006). Irradiating the sample with a laser converts the analyte into the gas phase and ionizes it. The sample is analyzed by rastering the laser over the tissue surface generating an ordered array of mass spectra which afford an ion density map. However, the MALDI matrix generates "background ions", which can interfere with the analysis, particularly, that of small molecules (Cohen & Gusev, 2002; Stump et al., 2002). A schematic of the process flow utilized for this report is depicted in Fig. 1. The aforementioned ionization methods are typically interfaced for ion detection with either