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Mass spectrometry imaging of levofloxacin distribution in TB-infected pulmonary lesions by MALDI-MSI and continuous liquid microjunction surface sampling

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ABSTRACT

A multi-modal mass spectrometry imaging (MSI) and profiling approach has been applied to assess the partitioning of the anti-TB fluoroquinolone levofloxacin into pulmonary lesions. Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) and a commercial liquid microjunction surface sampling technology (LMJ-SSP), or flowprobe, have been used to both spatially profile and image drug distributions in lung tissue sections from TB-infected rabbits following oral administration of a single human-equivalent dose.

Levofloxacin levels were highest at 6 h post-dose in normal lung, cellular granuloma, and necrotic caseum compartments. The drug accumulated in the cellular granuloma regions with lower amounts partitioning into central caseous compartments. Flowprobe imaging at 630 μm (limited by the probe tip diameter) enabled visualization of drug distribution into lesion compartments, including limited differentiation of relative drug abundance in cellular versus caseous regions of the lesions.

MALDI-MSI analysis at 75 μm provided more detailed drug distribution, which clearly accumulated in the cellular region immediately surrounding the central caseum core. Imaging and profiling data acquired by flowprobe and MALDI-MSI were validated by quantitative LC/MS/MS analysis of lung and granuloma homogenates taken from the same animals.

The results of the investigation show flowprobe imaging and sampling as a rapid and sensitive alternative to MALDI-MSI for profiling drug distributions into tissues when spatial resolution of data below the threshold of the probe diameter is not required.

1. Introduction

Mass spectrometry imaging (MSI) has been comprehensively demonstrated to be a powerful analytical technique for the localization of compounds within biological tissue sections [1–3]. It has several significant advantages to traditional whole-body autoradiography compound imaging, in that MSI does not require the synthesis of labeled compounds, which can prove costly and difficult to synthesize, and offers enhanced specificity as compounds and their metabolites are resolved through their specific mass [4]. However, WBA enables fully quantitative and (in most instances) higher spatial detail than MSI.

Of all the MS imaging modalities, MALDI has been the most applied technology for direct compound imaging in tissue [5–11]. Significant advantages include the sensitivity, potential high spatial resolutions (sub 10 μm [12]) and recently full on-tissue quantitation through the use of surface-spotted standards [13,14] or adjacently positioned spiked tissue homogenates to generate calibration curves [15]. However, the technology has a number of limitations, among which is ion suppression occurring due to the inherent heterogeneity of the tissue during direct tissue analysis. The lack of a chromatographic separation step during the MALDI-MSI experiment compounds this problem as the presence of heavily abundant species such as lipids and salts can result in a marked reduction in the detected ion signal for the compound of interest. Another important consideration in the sample preparatory steps is the ability to extract the compound of interest from the tissue and into applied surface matrix crystals without losing spatial integrity due to analyte delocalization. Careful optimization

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of any 'wet' matrix application technique must be performed as inhomogeneous extraction can lead to erroneous interpretation of ion images of drug distribution, particularly in heterogeneous tissue types such as tumors or lesions.

We have previously applied MALDI-MS imaging utilizing a QqQ mass spectrometer to image the distribution of Moxifloxacin (a synthetic broad-spectrum fluoroquinolone antibiotic) in rabbit lung biopsies from Tuberculosis (TB)-infected rabbits following oral dosing [8].

While MALDI has been the most commonly applied ionization method for drug and compound MS imaging in biological tissues, more recently, multiple alternative MS imaging modalities have emerged. Of particular interest are ambient electrospray ionization methods, which enable rapid, direct tissue imaging analysis outside of the vacuum chamber and without the requirement for time-consuming matrix application steps [16,17]. Of these techniques, Desorption Electrospray Ionization (DESI) is the most widely developed and has been applied to imaging of drugs, metabolites and lipids in a range of biological tissues [18–22]. Recently there has been increased adoption of methods for spatial profiling of analytes in tissue using liquid-microjunction based surface analysis. The in situ microextractions from these liquid microjunction surface sampling probes (LMJSSP) closely integrate sampling and ionization via liquid pumped to and aspirated away from the tissue surface prior to electrospray. The commercial and continuous iterations of these probes consist of two coaxial tubes, the internal of which is connected to the nebulizer at the site of electrospray to the MS inlet. Using the venturi created by the spray, the aspirated flow rate can be modified to balance the flow of solution pumped down the outer capillary, enabling a constant volume at the probe tip. When brought in proximity to the surface, this volume of extraction solvent forms a liquid microjunction between probe and sample, dissolving and entraining soluble analyte in the solution to be delivered to the ionization source. The technology has been applied to multiple surface analysis applications including thin layer chromatography plates, blood spots, microbial colonies and biological tissue sections [23–27]. The distribution of sulforaphane and its glutathione and N-acetyl cysteine conjugates was determined in whole body sections from a Sulforaphane-dosed mouse by selected sampling over the entire section. Whilst not generating a whole-body chemical image, this 'spatial profiling' method enabled rapid acquisition of localization information for both the drug and its metabolites without sample pre-treatment [23]. Recent enhancements include the incorporation of high-pressure liquid chromatography separation into the extraction workflow enabling isomeric drug metabolites to be resolved [28].

In this paper we present the comparative application of MALDI-MS imaging and a commercial LLMJSSP to profile and image the distribution of the fluoroquinolone antibiotic levofloxacin in lung biopsy sections from orally-dosed TB-infected rabbits. The ability of anti-TB drugs to penetrate into the pulmonary granuloma and central necrotic caseum is of critical importance as insufficient drug exposure to these areas can result in incomplete sterilization of resident bacteria and emergence of resistant mutants [29,30].

2. Method

2.1. Animal experiments and tissue collection

Experiments utilizing New Zealand White (NZW) rabbits were performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of Rutgers University under assurance #A-3158-01 and protocol #13034. Female rabbits used in infection studies were housed in individual cages in a biosafety level 3 (BSL3) animal facility approved for the containment of *Mycobacterium tuberculosis* (MTB).

Aerosol infection of rabbits was performed using a BioAerosol Nebulizing Generator (BANG) nebulizer delivering 18 L/min of filtered air and 6.4 L/min of aerosol (2.5×10^6 CFU/L in phosphate-buffered saline) to the CH Technologies inhalation system (Westwood, NJ). The infection was allowed to develop for 16–21 weeks prior to drug administration, by which time numerous (>50) granulomas with diverse pathology (cellular, necrotic, caseating and fibrous) could be harvested from the lungs.

Rabbits were dosed by oral gavage with levofloxacin (Sigma, St Louis, MO) at a final concentration of 75 mg/kg, the human-equivalent dose. The animals were randomly assigned to necropsy at 2 h, 6 h or 24 h after drug administration. For MS imaging experiments, small pieces of lung tissue containing a minimum of one well-developed necrotic lesion were excised and immediately flash frozen in liquid nitrogen vapor. Samples for LC-MS/MS drug quantitation were removed and prepared as previously described [8].

All MTB infected rabbit tissues were processed in a certified BSL3 facility until the viable micro-organisms had been inactivated. Sterilization of samples for imaging studies was performed by γ-irradiation. Rabbit lung biopsies were arranged in a single vertical layer in dry ice and exposed to γ-irradiation in a 60Co irradiator using the nearest position and all three rods until 3 MRad was delivered. The procedure was validated internally to demonstrate that all MTB bacilli are killed upon delivery of such dose of γ-rays.

2.2. Tissue sectioning and matrix application

Twelve micrometer thick tissue sections were prepared using a Leica CM1850 cryostat (Buffalo Grove, IL) and mounted onto stainless steel slides (for MALDI-MSI analysis) or frosted glass microscope slides (for flowprobe imaging, profiling and histology). After sectioning, tissue sections were immediately transferred to a −80 C freezer for storage.

Prior to MALDI-MSI analysis, tissue sections were removed from the −80 °C freezer and allowed to reach room temperature for 15 min. Three milliliter of 50% methanol containing 2 pmol/μL levofloxacin-d_3 (C/D/N Isotopes, Quebec, Canada) was applied to the surface by airspray deposition at 40 psi, followed by 25 mg/mL DHB (50% methanol, 0.1% TFA). The airbrush (Paasche Model VL, Chicago, IL) was positioned at a distance of 30 cm from the tissue and 20 passes over the tissue were performed with the tissue being allowed to fully dry between coatings. This approach was chosen as applying the internal standard independently of the matrix application has been shown to produce a more homogeneous signal for normalization purposes [31]

2.3. MALDI-MSI analysis

Optimization of MALDI Orbitrap XL instrument parameters was performed by spiking 1 μl of a 10 pmol/μL levofloxacin standard (in 50% methanol) onto the surface of 12 μm thick control rat lung sections. DHB (25 mg/mL in 50% methanol) was applied by airspray as described in the previous paragraph. Laser energy, number of laser shots, and number of microscans were selected to maximize signal to noise for the levofloxacin *m/z* 362.150 peak and the deuterated levofloxacin standard at *m/z* 365.168.

MALDI-MSI analysis was performed using a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with a resolution of 60,000 (at *m/z* 400, full width half maximum (FWHM)). The resolution was sufficient to resolve the desired levofloxacin and levofloxacin-d3 peaks from background without the requirement for MS/MS and subsequent loss of signal.

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