Utility of high resolution MALDI imaging in drug discovery:
Histological distribution of gentamicin in proximal renal tubules of rats

Overview
- MS/MS imaging of gentamicin was performed from high-selectivity analysis of endogenous metabolites in biological tissue.
- High resolution (10 µm) imaging of rat renal cortex showed that gentamicin is specifically distributed in proximal renal tubules.
- New matrix application method is useful for the sensitive MALDI imaging.

1. Introduction
In pharmacology and toxicology, localization of a drug molecule in the target tissue of organs provides very important in vivo biological information. Imaging mass spectrometry (IMS) is increasingly used in drug discovery and development during preclinical studies. Gentamicin is an antibiotic to treat infections, but it possesses renal toxicity. As it is excreted in the urine, the kidney tissues of a person being treated with gentamicin are almost constantly bathed in gentamicin. The objective of the study is to define the specific distribution of the parent drug in proximal renal tubules of rats dosed gentamicin using MALDI imaging.

2. Methods
At 2 h after single intravenous (I.V.) administration (3, 10, or 30 mg/kg) of gentamicin to male SD rats, 5-µm sections taken from the single kidney of each rat were prepared. The tissue sections were coated with CHCA by sublimation using an automated sample treatment system (iMLayer™, Shimadzu Corporation, Japan), and analyzed using an ion trap-time of flight (IT-TOF) tandem mass spectrometer equipped with MALDI source†. This instrument is a combination of an optical microscope which allows the observation of high-resolution morphological images, with a mass spectrometer which identifies and visualizes the distribution of specific molecules. The other kidney was used for determining the concentrations of gentamicin by LC-MS/MS.
3. Results

Data acquisition was performed in product ion scan (MS/MS) mode and abundance of gentamicin C1 was selectively monitored by focusing on its specific fragment ion, \( m/z \) 322.196.

**Optimization of matrix application method**

1) Spray

2) Sublimation + Spray

3) Sublimation + Nebulizer\(^*\)

4) Pre-coated matrix + Sublimation + Nebulizer


The peak intensities of gentamicin C1 by Method 4) rose 20 times from those by Method 1). \( m/z: 478.3 \rightarrow 322.2 \)
Abundance of gentamicin C₁ was higher in the renal cortex than in the renal medulla. The signal intensities of m/z 322 of gentamicin within the kidney in distribution images corresponded well with the gentamicin concentrations.

MS/MS spectrum of gentamicin C₁ in rat kidney dosed 30 mg/kg of gentamicin
Precise Localization of Gentamicin in Renal Cortex

The image of the specific distribution of gentamicin in proximal renal tubules of rats was detected with a high resolution of 10 µm (pixel size), and found to be similar to the distribution of the immunostaining assay of gentamicin reported previously*.


4. Conclusions

High resolution (10 µm) imaging of rat renal cortex showed that gentamicin is specifically distributed in proximal renal tubules. Research areas and target compounds involved in pharmaceutical drug discovery is very wide ranging. Imaging mass spectrometry using Mass Microscope is one powerful tool for the drug discovery (pharmacokinetics, pharmacological mechanisms, toxicity), owing to its ability to easily combine morphological observation from the optical microscope and localization of target molecules from the mass spectrometer image.

5. Acknowledgments

This work was supported by Safety Research Laboratories of Mitsubishi Tanabe Pharma Corporation.

* The MS instrument used in this poster is currently not for sale in the United States.