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Application News

No. B61

Imaging Mass Microscope

A Study of Toxicity Evaluation Using the iMScope *TRIO* - Analysis of Localization of Amiodarone in Rat Lungs -

In drug discovery research, the analysis of the pharmacokinetics of candidate compounds provides important information not only in the elucidation of pharmacological mechanisms, but also from the viewpoint of toxicity assessment. In general, a method using autoradiography (ARG) and fluorescent dye is used, but with ARG the costs are high, and there have been concerns about the effects of using fluorescent agents as labeling agents on the pharmacokinetics. In recent years, the analytical technique of MS imaging has been attracting attention as a method that can detect data on the localization of candidate compounds without using a label. This method is expected to provide a breakthrough in drug discovery research as it can be used to analyze the localization of various substances without labeling, and to simultaneously analyze the unchanged drug and its metabolites using the same section.

Here, we introduce an example of MS imaging analysis using the iMScope *TRIO* imaging mass microscope (Fig. 2) to compare the localization of the pathological findings with that of the amiodarone observed in lung tissue after administering amiodarone (Fig. 1) to rats.

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Analysis of Localization of Amiodarone in Rat Lungs

In this experiment, we measured the tissue sections of rats lungs that had been administered amiodarone, an anti-arrhythmia drug. When administered in large quantities, amiodarone causes phospholipidosis and pathological findings such as foamy macrophage infiltration of cells are observed. However, up until now there had been no information on whether amiodarone accumulated in the lesions or not, so we examined the relationship between the pathological findings and localization of amiodarone utilizing the MS imaging technique. In the preliminary study test using standard amiodarone, we optimized the matrix selection and measurement mode, and applied those conditions in this experiment. Table 1 shows the experimental conditions from sample preparation to MS imaging analysis.

On comparing the mass spectrum taken from a tissue section obtained by performing high-resolution imaging with a spatial resolution of 5 µm using the iMScope *TRIO* with the mass spectrum of the standard, a common *m/z* 646.0 signal was detected (Fig. 3). By drawing a mass image of this *m/z* 646.0, we confirmed that amiodarone had accumulated where foamy macrophages had infiltrated the cells (Fig. 4).

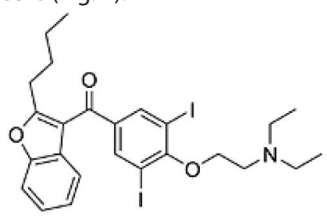


Fig. 1 Structural Formula of Amiodarone

Fig. 2 iMScope *TRIO*

Table 1 Experimental Conditions

Sample Preparation		
	Animal species	: Rat
	Administered drug	: Amiodarone hydrochloride
	Administration method	: 3-day repeated oral administration
	Dose	: 1000 mg/kg
	Organ	: Pulmonary tissue
	Section	: Fresh frozen sections
	Section thickness	: 10 µm
Matrix Coating		
	Matrix	: CHCA
	Matrix coating method	: Sublimation by iMLayer
	Matrix coating thickness	: 0.7 µm
Measurement Conditions		
	Analysis instrument	: iMScope *TRIO*
	Measurement mode	: positive mode
	MS range	: *m/z* 500-700
	Laser diameter	: 5 µm
	Spatial resolution	: 5 µm

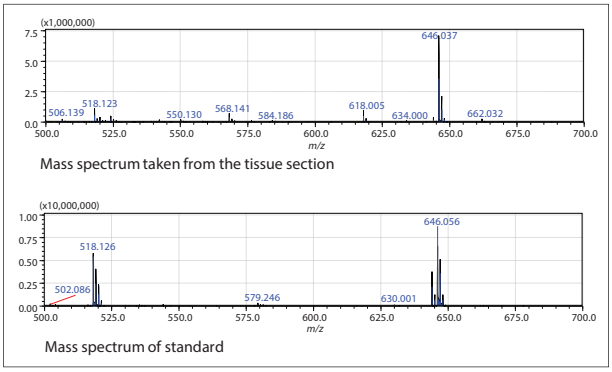
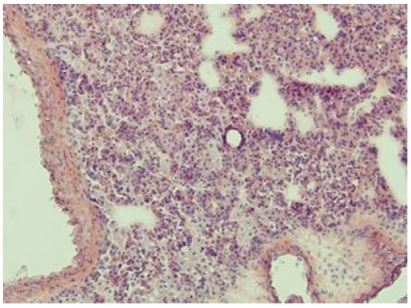
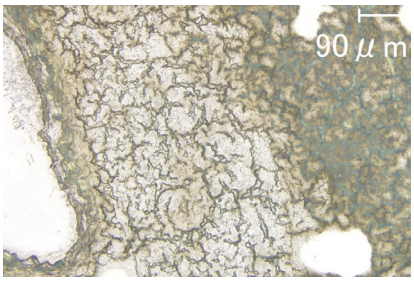


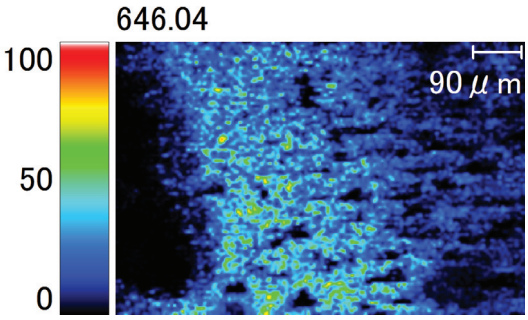
Fig. 3 Mass Spectra of the Tissue Section and Standard



Section of lung tissue with amiodarone administered (stained with HE)



Section of lung tissue with amiodarone administered (Unstained for MS imaging analysis)



MS image (unchanged amiodarone)

Fig. 4 HE-Stained Image and Optical Image for Analysis (Serial Sections) and MS Image

Analysis of Localization of Amiodarone Metabolites

In rats, administered amiodarone is reported to be N-deethylated in the body. In this experiment we performed MS scan analysis, and a strong peak was also observed 28 Da lower, at *m/z* 618.0, corresponding to deethylation of unchanged amiodarone (Fig. 5). By drawing the MS image for *m/z* 618, we obtained an image similar to that of the localization of amiodarone, as shown in Fig. 6. This also indicates a high probability that the MS image of *m/z* 618.0 depicts a product of N-deethylation of amiodarone.

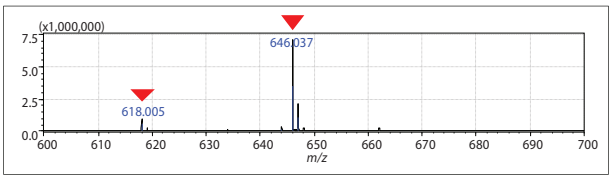


Fig. 5 Mass Spectrum of Tissue Section (Detail of *m/z* 600 to 700 Range)

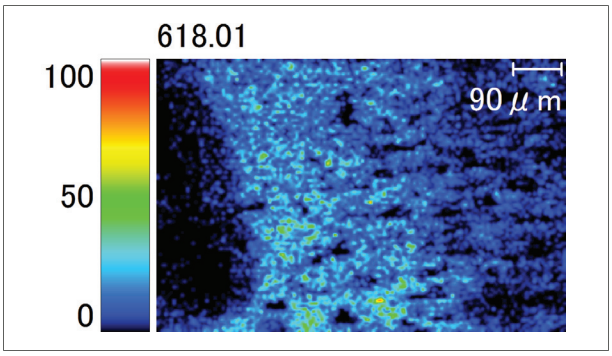


Fig. 6 MS Image (Corresponding to Molecular Weight of Metabolite)

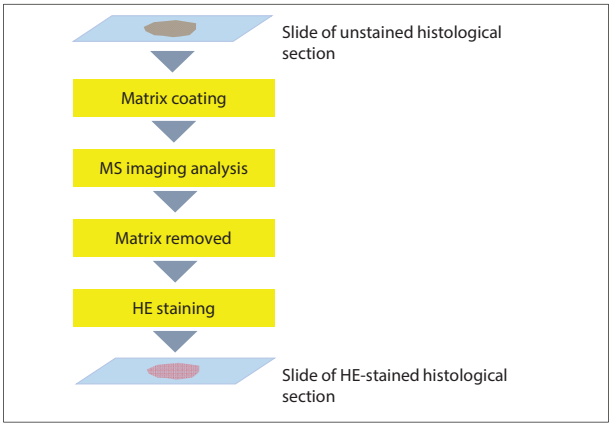


Fig. 7 Workflow for HE Staining After MS Imaging Analysis

Prospects in Perfectly Matching MS and HE-Stained Images

Since MS imaging is not possible on a section stained with HE, in this experiment MS imaging analysis was performed on an unstained section that was consecutive to the one stained with HE. However, even with serial sections the tissue morphology is only similar and not a perfect match, and the images have to be aligned by relying on distinctive landmarks. Currently, in order to solve this problem, we are considering perfectly matching the position information of the HE-stained image and MS image by removing the matrix from the section that has been subjected to MS imaging with an organic solvent and then staining it with HE, as shown in Fig. 7.

Acknowledgments

We would like to thank Mr. Hidefumi Kaji and Mr. Hiroyuki Hashimoto of Mitsubishi Tanabe Pharma Co., Ltd. very much for their great cooperation with obtaining data for this Application News and the writing of this article.

First Edition: Jun. 2017

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