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Rapid pharmacokinetic analysis of small drugs by mass spectrometry imaging combined with product ion analysis

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1. Abstract

Introduction: Comprehensive information of administered drugs in biological tissues or organs is of paramount importance to understand their clinically-relevant properties, efficacy, potential side effects, and toxicity. Mass spectrometry imaging (MSI) is a label-free and highly sensitive imaging technique that offers accurate visualization of drugs and their metabolites in the tissue sections. Among several tools of MSI, matrix-assisted laser desorption/ionization MSI (MALDI-MSI) is most commonly used for mapping of drugs, their metabolites, and other analytes present in the sample. In this study, we are using a newly developed MALDI-MSI tool known as iMScope^{TM} QT for rapid pharmacokinetics analysis of imipramine and chloroquine.

Methods: Thirteen weeks old C57BL/6 male wild type (WT) were treated (intraperitoneal injection) with imipramine and chloroquine (30 mg/kg) for pharmacokinetics analysis of these drugs. After two hours of injecting drugs, all major organs were collected following cervical dislocation. Thereafter, 10 µm section from mice kidney, brain, and liver were prepared and MSI data were acquired applying a quadrupole time-of-flight mass spectrometer equipped with an atmospheric pressure MALDI source (iMScope^{TM} QT, Shimadzu, Japan). Prior to acquire MSI data, MALDI-MSI methods were optimized for rapid (9-10 spectra/sec) imaging of imipramine, chloroquine and their metabolites with higher sensitivity at higher spatial resolution. Now we are trying to apply product ion analysis (MS/MS) for better confirmation of drugs and their metabolites detected by iMScope QT. This study is authorized by the ethics committee of Hamamatsu University School of Medicine, Shizuoka, Japan.

Results: Applying iMScope QT, we revealed the localization of imipramine, chloroquine, and their metabolites in the different organs of mice for the first time. In our current study, we observed that the imipramine and its metabolites were highly accumulated in the upper medulla of mice kidney, where chloroquine and its metabolites were significantly accumulated in the pelvis and inner medulla of mice kidney. Additionally, higher accumulation of imipramine were noted in the hindbrain, midbrain, thalamus, hypothalamus and septum of the brain of treated mice. Chloroquine and its metabolites were also detected throughout the brain of treated mice, however their notable accumulation were observed in the lateral ventricle, 4th ventricle, and fornix of the brain of treated mice. These drugs and their metabolites were also successfully detected in the liver of treated mice by iMScope QT. Now we are preparing mice with different doses and treatment period for the detailed pharmacokinetics analysis of these drugs in all major organs of mice applying iMScope QT.

2. Materials and Methods

Method optimization: To confirm the detection of targeted drugs and their metabolites with higher sensitivity at higher spatial resolution, we tried three matrix, 2,5-Dihydroxybenzoic acid (DHB), α-Cyano-4-hydroxycinnamic acid (α-CHCA) and 9-aminoacridine (9AA). For matrix application, we tried iMLayer^{TM} (Shimadzu) and TM-Sprayer^{TM} (HTX Technologies). Depending on our observation, application of 0.7 µm thick α-CHCA matrix using iMLayer was chosen for better ionization of targeted analytes and higher spatial resolution.

Preparation of mice sample: In our preliminary experiment, WT mice were injected with imipramine and chloroquine dissolved in normal saline water at the dose of 30 mg/kg of body weight. After two hours, all mice were sacrificed following cervical dislocation and all major organs were collected. After making freeze block, 10 µm sagittal sections were prepared and mounted on ITO coated glass slide (100 Ω).

Matrix application: α-CHCA was heated at 250 °C and deposited on the specimen surface (0.7 µm) applying iMLayer. The iMLayer chamber was maintained at vacuum condition during the matrix deposition (Fig. 1A)

Data acquisition applying iMScope QT: Following parameters were used to acquire data by iMScope QT (Fig. 1B) from mice samples; polarity: positive, *m/z* range: 150 to 450, pixel size: 50 µm x 50 µm (X x Y), laser diameter: 25 µm, laser power: 70, laser shots: 100, repetition rate: 1000 Hz, detector voltage: 2.4 kV, DL temperature: 300 °C.

Data analysis: iMScope QT data were analyzed using IMAGEREVEAL^{TM} MS software (Shimadzu, Japan).

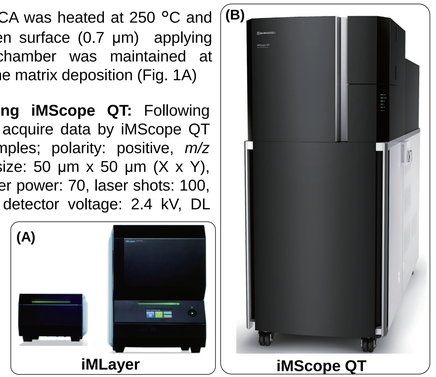

Figure 1: Representative images of iMLayer and iMScope QT.

3. Results and Discussion

3-1. Detection of imipramine, chloroquine and their metabolites by iMScope QT

After optimizing matrix thickness and iMScope QT parameters, data were acquired from mice kidney, brain and liver sections, and standard drugs by iMScope QT. We successfully detected both imipramine, chloroquine and their several metabolites from the kidney, brain and liver of treated mice (Fig. 2 and Table 1). Compared to conventional MSI, we acquired data rapidly with iMScope QT (9-10 spectra/sec).

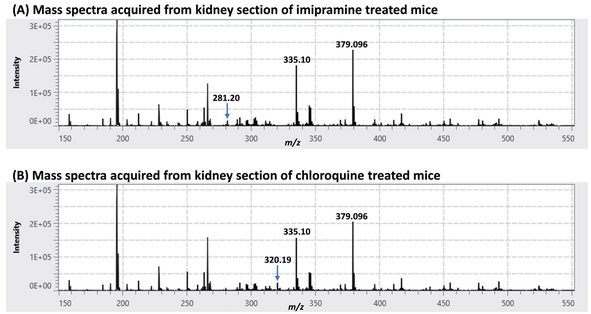

Figure 2: Representative mass spectra acquired from the sagittal sections of imipramine and chloroquine treated mice kidney. *m/z*: mass to charge ratio; *m/z* 281.20: imipramine as protonated adduct, *m/z* 320.19: chloroquine as protonated adduct.

Table 1: List of the metabolites of imipramine and chloroquine detected by iMScope QT from the kidney, brain and liver sections of treated mice.

Imipramine and its metabolites					
Candidates	**Molecular formula**	**Monoisotopic mass**	**Kidney**	**Brain**	**Liver**
Desdimethyl-imipramine	$C_{17}H_{20}N_2$	252.1626	Detected $[M+Na]^+$	Detected $[M+Na]^+$	Not detected
Desipramine	$C_{18}H_{22}N_2$	266.1783	Detected $[M+H]^+$	Detected $[M+H]^+$	Detected $[M+H]^+$
Imipramine	$C_{19}H_{24}N_2$	280.1939	Detected $[M+H]^+$	Detected $[M+H]^+$	Detected $[M+H]^+$
2-hydroxyimipramine	$C_{19}H_{24}N_2O$	296.1889	Detected $[M+H]^+$	Not detected	Not detected
2-hydroxy-imipramine glucuronide	$C_{25}H_{32}N_2O_7$	472.2209	Detected $[M+H]^+$	Not detected	Not detected
Chloroquine and its metabolites					
Candidates	**Molecular formula**	**Monoisotopic mass**	**Kidney**	**Brain**	**Liver**
Chloroquine-M(-N(C_2H_5)$_2$)	$C_{14}H_{18}ClN_2$	246.0924	Detected $[M+H]^+$	Not detected	Not detected
Bisdesethylchloroquine	$C_{14}H_{18}ClN_3$	263.1189	Not detected	Detected $[M+H]^+$	Not detected
Desethylchloroquine	$C_{16}H_{22}ClN_3$	291.1502	Detected $[M+H]^+$	Detected $[M+H]^+$	Detected $[M+H]^+$
Chloroquine	$C_{18}H_{26}ClN_3$	319.1815	Detected $[M+H]^+$	Detected $[M+H]^+$	Detected $[M+H]^+$
Chloroquine di-N-oxide	$C_{18}H_{26}ClN_3O_2$	351.8716	Not detected	Detected $[M+Na]^+$	Not detected

3-2. Localization of both drugs and their metabolites in mice kidney

Imipramine, chloroquine and their metabolites were successfully detected in the kidney of treated mice. Imipramine and its metabolites desipramine, 2-hydroxyimipramine, and desdimethyl-imipramine were notably accumulated in the upper medulla and cortex of mice kidney, but 2-hydroxyimipramine glucuronide was highly accumulated in the inner medulla and pelvis of mice kidney (Fig. 3A). Higher accumulation of chloroquine and desethylchloroquine was found in the pelvis and medulla of mice kidney, but chloroquine-M(-N(C_2H_5)$_2$) was detected in the cortex and outer medulla of mice kidney (Fig. 3B).

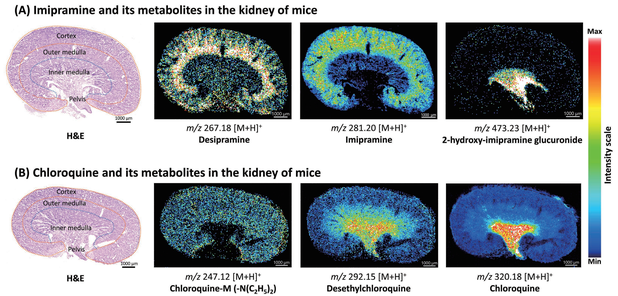

Figure 3: Spatial distribution of imipramine, chloroquine and their metabolites in kidney.

3-3. Localization of both drugs and their metabolites in mice brain

Both drugs and their metabolites were also successfully detected in the brain of treated mice. Although imipramine and its metabolites were observed throughout the brain, higher accumulation of imipramine was noted in the hindbrain, midbrain, thalamus, and fornix (Fig. 4A). Similar to imipramine, chloroquine and its metabolites were detected throughout the brain, but notably accumulated in the lateral and 4th ventricle of treated mice brain (Fig. 4B).

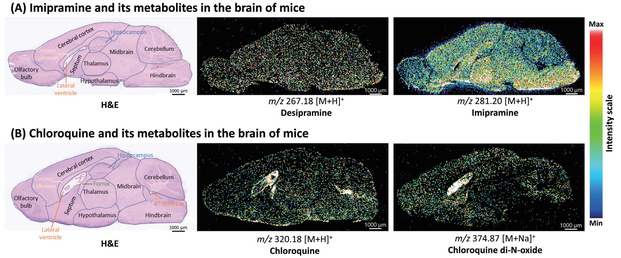

Figure 4: Spatial distribution of imipramine, chloroquine and their metabolites in brain.

3-4. Imipramine, chloroquine and their metabolites detected in liver

Both drugs and their metabolites were also detected in the liver of treated mice. Among imipramine and chloroquine, chloroquine and its metabolites showed higher accumulation in the liver of treated mice (Fig. 5).

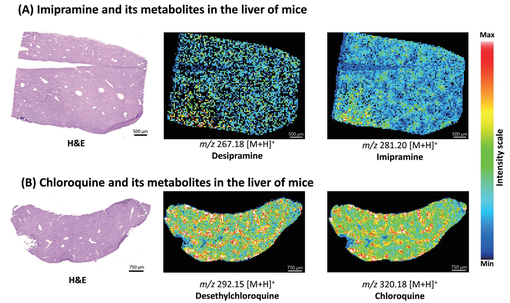

Figure 5: Ion images of imipramine, chloroquine and their metabolites detected in liver.

4. Conclusions

In our preliminary study, we have revealed specific localization of imipramine, chloroquine, and their metabolites in different organs of mice for the first time applying a newly developed tool of MSI named iMScope QT. Now we are preparing samples with different doses and time-points to explore detailed pharmacokinetics of these drugs in mice. We hope that out work will be helpful to understand detailed metabolism, function and side effects of those drugs.

5. Funding

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