Visualization of Intact protein for the study of lithium neuropharmacology in mouse brain with MALDI - Imaging Mass Spectroscopy

Genomics, Proteomics and Biomedical Functions
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Introduction

Lithium (Li) is a well-established therapeutic drug for bipolar disorder and major depression. More recently, Li has also been regarded as a neuroprotective agent and a candidate drug for disease-modification in certain neurodegenerative disorders such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD).

While the putative neuroprotective effects of Li are extensively studied through in vitro study, however, an exact pharmacokinetics and pharmacodynamics (PKPD) of Li was not clear. In a murine model of Li treatment, here we use MALDI Imaging mass spectrometry (IMS) to delineate Li administration effects of the brains at proteomic level. Li distribution in the brains was also monitored by LA ICP-MS and TOF-SIMS.

Methods

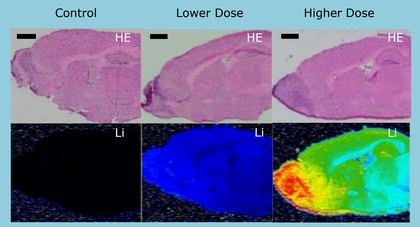
Animals: C57BL/6 mice at 8 weeks of age were fed for this study for 14 days. Experiments were performed using procedures approved by the Experimental Animal Research Committee at the Doshisha University.

Mice had free access to Li containing water. Three different dose of Li were administered as 0 mM (Control), 4.72 mM (Lower Dose) and 14.2 mM (Higher Dose). Lithium in Tap Water were fed for 14 days. Mice were sacrificed by decapitation following isoflurane anesthesia at day 14. Brain was removed and storage by -80℃.

Tissue preparation: Frozen tissue sections were cut on a cryostat at a 10 mm thickness for MALDI-Imaging were obtained.

MALDI-Mass Imaging: Images were acquired using the rapifleX MALDI Tissuetyper, at a spatial resolution of 50 μm. Visualization and statistical analysis were performed by FlexImaging and SciLS Lab 2016a.

Visualization of Li in mouse brains

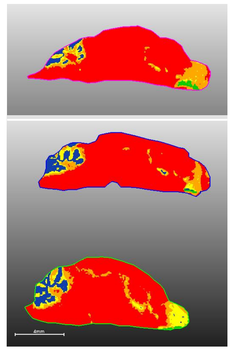


Upper:HE staining, Lower:IMS of Li (Calibration by 13C)

By using LA-ICP-MS, we visualized pharmacologically administrated Li distribution in dose dependent manner. It was between 0.87 to 1.10 mm apart from the mid brain. Especially in Higher Dose, Li concentrated at Olfactory Bulb (OB) and Hippocampus (HP).

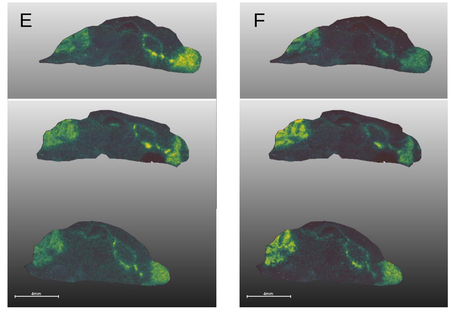
Results

- Segmentation map



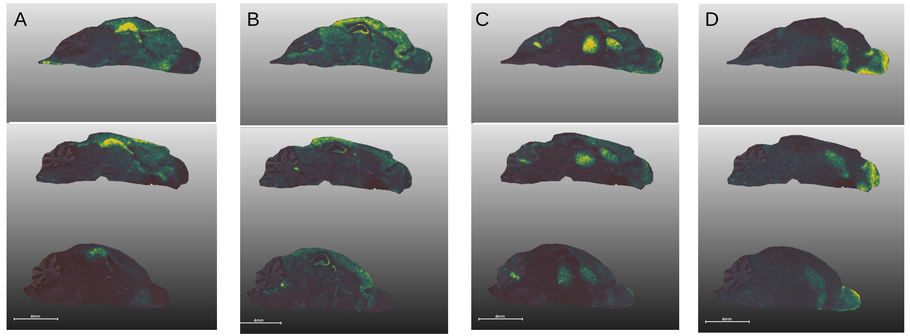
Segmentation map of the brains from Li-treated mice. Top; Control, Middle; Lower Dose of Li, Bottom; Higher Dose of Li administration. Bar = 4 mm. While structure of the cerebellum was uniformly segmented, Rostral migratory stream (RMS) and Main Olfactory Bulb (MOB) area was strengthened with Li administration.

- Single mass imaging



Functional proteins : Intact proteins at m/z 7044 (E) and m/z 14047 (F) were visualized. These proteins were detected at main olfactory bulb (MOB), rostral migratory stream (RMS), and cerebellum (Cb). Intensity of the proteins at OB may be influenced by Li administration. Top; Control, Middle; Lower Dose of Li, Bottom; Higher Dose of Li administration. Bar = 4 mm.

- Single mass imaging



Morphological proteins: Intact proteins at m/z 4976.994 (A), 6294.189 (B), 6738.301 (C), 7433.487 (E) were visualized. These proteins were detected in an area specific manner. (A) (B) Hippocampus and Cortex (C) Caudaputamen and thalamus, Cortex, Cerebellum (D) Thalamus and olfactory bulb, glomerular area. Top; Control, Middle; Lower Dose of Li, Bottom; Higher Dose of Li administration. Bar = 4 mm.

Conclusions

- In mouse brains, Lithium administration was monitored through LA-ICP-MS, which is detected mostly in olfactory bulb in a dose dependent manner.
- Brains from Li-treated mice were analyzed through MALDI-IMS at intact proteome level.
- Effects of Li-treatment on mouse brains were successfully visualized with segmentation map as well as single mass imaging of proteins morphologically and functionally detected.

Imaging Mass Spectrometry