Visualization of metabolites in the central nervous system (CNS) of murine olfactory deprivation model with MALDI Mass Spectrometry Imaging

Genomics, Proteomics and Biomedical Functions
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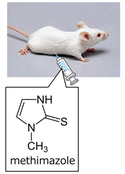
Introduction

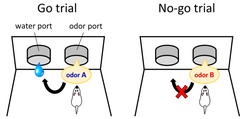
Olfactory dysfunction is among the earliest features of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Although neuropathological abnormalities have been detected in the olfactory bulb (OB) for both diseases, little is known about its dynamic biology. Here we generate murine olfactory deprivation model by single intraperitoneal administration of Methimazole (MTZ), a drug for hyperthyroidism with a unique side effect of temporary and reversible olfactory epithelium (OE) depletion. For this experiment, we have defined global metabolomics changes of the murine central nervous system (CNS) using MALDI mass spectrometry imaging (MSI).

Method

Animals:
Experiments were performed using procedures approved by the Experimental Animal Research Committee of Doshisha University. Intraperitoneal MTZ administration (75mg/dl i.p.) were performed for 8-week-old ICR female mice.



Behavioral Testing:
The Go/No-Go odor discrimination task was performed on a mouse model of olfactory dysfunction using p-Cresol as OdorA and Cuminaldehyde as OdorB. After 2 weeks of training, the MMZ group was treated with Methimazole and the Cont group with saline. Then the percentage of correct responses was determined. Statistical processing was performed using F-test and t-test or U-test.

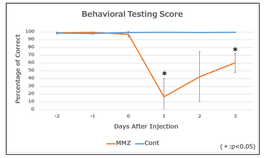


Tissue preparation:
Frozen tissue sections of the mice were cut to 10μm thickness in a cryostat. HE staining and TUNEL staining were obtained.

MALDI-Mass Imaging:
Spectra were acquired using timsTOF flex and ions were detected with a spatial resolution of 50μm. Used matrix was 2,5-Dihydroxybenzoic Acid (DHB) and derivatization agent (Py-Tag 0) sprayed with TM-sprayer. FlexImaging and SCiLS Lab 2022b were used for visualization and statistical analysis.

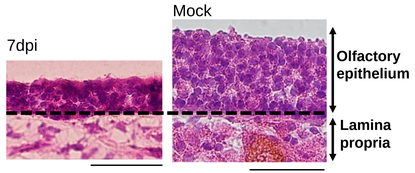
Result and Discussion

MTZ-induced olfactory epithelium damage



The percentage of correct responses was significantly decreased even on the 3rd day after administration, when the number of tasks in the mice had recovered to the same level as before administration.

MTZ-induced olfactory epithelium damage



HE staining of the olfactory epithelium of MTZ-treated mice, we confirmed that the thickness of the mouse olfactory epithelium became thinner on day 7 of treatment against Mock.

Mapping metabolites on olfactory dysfunction model

Sagittal sections of the OB-Rostral Migratory Stream (RMS)-Subventricular zone (SVZ); in one section of murine olfactory deprivation models were analyzed using timsTOF fleX (Bruker Daltonik GmbH). Serial sections were stained by Hematoxylin and Eosin stain.

Figure.1

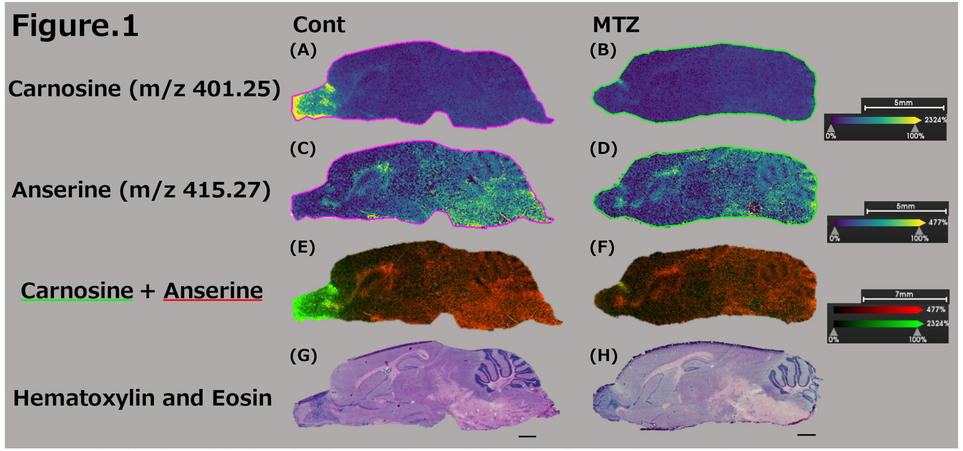


Figure.1 Distribution of derivertized Carnosine and Anserine in the Mouse Brain
Single mass images of derivertized Carnosine (A,B) and Anserine (C,D) are visualized. (Bar = 5mm). (A) In Cont, Carnosine showed the strongest peak intensity in the Glomerular Layer (GL) of the Olfactory Bulb (OB). Peaks were also observed in the External Plexiform Layer (EPL), Mitral Cell Layer (MCL), and Granule Cell Layer (GCL). (B) In contrast, no such peak was observed in MTZ. (C,D) Anserine, one of the same imidazole dipeptides as Carnosine, showed no significant difference between Cont and MTZ.(E,F) Merged images of Carnosine and Anserine single peak images. (green: Carnosine, orange: Anserine) (G,H) HE stained image of each sections.(Bars = 1mm)

Conclusion

- MALDI-IMS is a useful approach in understanding effects of olfactory deprivation of mice at metabolomic level.
- We found the candidate metabolite for further analysis of molecular mechanisms for the studies of olfactory related neurological disorders.

Acknowledgements

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Imaging MS:
Pharmaceuticals, Metabolites, Lipids and Glycans