Distinct deposition of amyloid-β species in brains with Alzheimer's disease pathology visualized with MALDI imaging mass spectrometry

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overview

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Introduction

Alzheimer's disease (AD) represents the most common age-related neurodegenerative disorder and cause of dementia worldwide. Individuals afflicted with AD harbor two pathological signatures within their brains: extracellular amyloid plaques and neurofibrillary tangles (NFTs), which are identifiable only by post-mortem examination. The formation of plaques containing a high concentration of amyloid β (Aβ) peptides is thought to play a central role in the disease's pathogenesis. These deposits are mainly composed of peptides of 4–5 kDa molecular mass, all derived from amyloid precursor protein (APP). In this study, we applied matrix-assisted laser desorption/ionization (MALDI) mass imaging mass spectrometry (IMS) for human autopsied brain samples as well as conventional immunohistochemistry. Here we demonstrate a detailed analysis of the different variants of Aβ peptides in the brains from patients with and without AD pathology including a variety of cerebral amyloid angiopathy (CAA).

Methods

Subjects: Human cortical specimens for IMS and immunohistochemistry were obtained from those brains that were removed processed and placed in -80˚C within 8h postmortem at the Brain bank at Tokyo Metropolitan Institute of Gerontology. For all brains registered at the brain bank we obtained written informed consents for their use for medical research from patients or patient's family. Each brain specimen was taken from occipital cortex of 5 AD patients and 5 controls.

case	gender	age at death	Braak Stage	CAA
1	M	83	5	0.5
2	M	88	5	1
3	M	84	5	2
4	M	78	6	1
5	M	83	5	1
6	M	84	1	0
7	M	78	1	0
8	M	70	1	0
9	M	73	1	0
10	M	81	1	0

Table 1 Clinical and pathological data of AD with CAA cases and control

MALDI Imaging: Frozen tissue sections were cut on a cryostat (CM1950, Leica Microsystems, Wetzlar, Germany) at a 10 μm thickness onto indium-tin-oxide–coated glass slides (Bruker Daltonics, Bremen, Germany). Before matrix coating, treated with a formic acid. For mass spectrometric measurements, tissue areas were defined using the FlexControl 3.8 and FlexImaging 5.0 software packages (both Bruker Daltonics). Spectra were acquired using the rapifleX MALDI Tissuetyper (Bruker Daltonics) in positive linear mode, whereas ions were detected in a mass range of m/z 2,000 to 20,000 with spatial resolution of 20, 50 and 100 μm. A ready-made protein standard was used for spectra calibration (Bruker Daltonics). Visualization and statistical analysis were used FlexImaging and SciLS Lab 2016a (SCiLS, Bremen, Germany).

Immunohistochemistry: Fresh frozen sections were post-fixed by 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). After rinses with PBS-T, bound antibodies were visualized with secondary antibodies conjugated with Alexa dyes (life technology). The specimens were analyzed by confocal-laser-scanning-microscope (LSM700; Carl Zeiss Inc.)

Results and Discussions

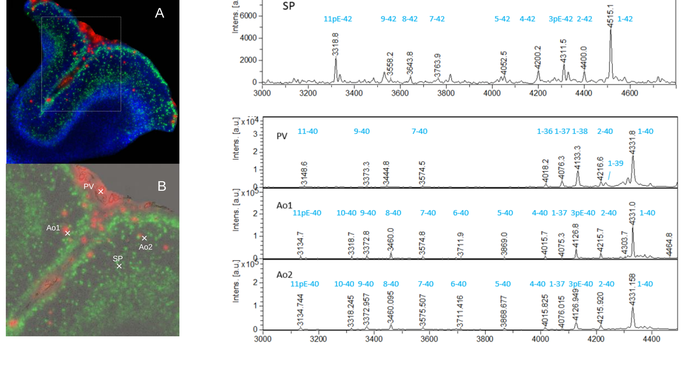


Figure.1 MALDI Imaging Mass Spectrometry for AD brain. Subject #1 and #2. A: Aβ1-40 deposited in leptomenigeal and parenchymal arterioles (red) and Aβ1-42 deposited in cerebral parenchyma (green) respectively. B: This figure comes from square of Fig. 1A. Aβ1-40 deposited in perivascular vessels (PV) and arterioles (Ao) as shown red. Aβ1-42 deposited in cerebral parenchyma as senile plaques (SP) as shown green. C: MALDI Mass spectrum in SP, PV and Ao 1 and 2 respectively. Aβ1-42 and N-terminal truncated forms were in SP. Aβ1-36, 37, 38, 39, and 1-40 in addition to N-terminal truncated forms of Aβ40 were in PV and Ao. D: N-terminal truncated and modified Aβs were detected with IMS.

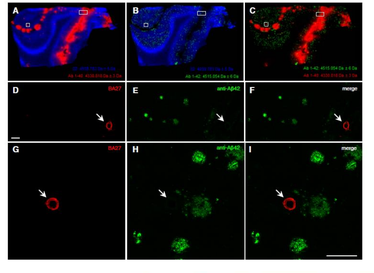
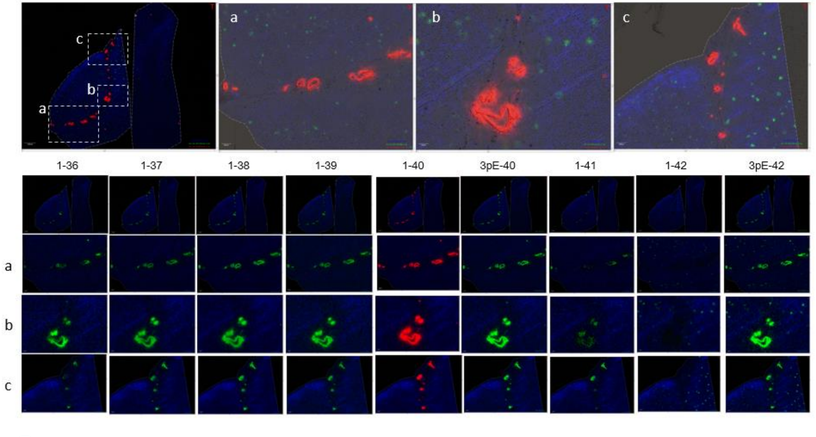


Figure.2 MALDI Imaging Mass Spectrometry and Immunohistochemistry for AD brain with CAA. Adjacent sections of the occipital cortex from AD brains were subjected immunostaining using antibodies of Aβ40 (BA27) or Aβ42 (anti-Aβ42 polyclonal) and merged view. IHC analysis also demonstrated the differential distribution of Aβ40 and Aβ42, CAA dominant and SP dominant deposition respectively. Dashed and solid rectangles indicate the area demonstrated in panel respectively. Bars = 100 μm. Green: Aβ 1-42, Red: Aβ 3pE-42. C,D: N-truncated/modified Aβ40 (C) and Aβ42 (D).

Figure.3 IMS for frozen AD/CAA brain sections (No.3) at higher resolution. Various C-terminal and N-terminal truncated and modified Aβ peptides in AD accompanying severe CAA (NO. 3). Aβ1-36 to Aβ1-41 are preferentially deposited in leptomeningeal blood vessels, while Aβ1-42 and Aβ1-43 are deposited in the cerebral parenchyma as senile plaques. Resolution = 20 μm.



Summary

- **MALDI-IMS clarifies Aβ distributions in brains with AD / CAA**
- **Detecting Aβ 1-41 through MALDI-IMS in consistent with IHC**
- **MALDI-IMS shows distributions of N-truncated peptides of Aβ 40 and Aβ 42**

Reference

Kakuda N and Miyasaka T et al (2017) Distinct deposition of amyloid-β species in brains with Alzheimer's disease pathology visualized with MALDI imaging mass spectrometry. ***Acta Neuropathologica Comm***. 5: 73. Oct 16.