Abstract of Dissertation submitted by DONG THI THU TRANG

Title: Formalin RT-QuIC assay detects prion-seeding activity in formalin-fixed brain samples from sporadic Creutzfeldt–Jakob disease patients
ホルマリン RT-QuIC 法は孤発性クロイツフェルト・ヤコブ病患者のホルマリン固定後脳からプリオンシード活性を検出できる

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Introduction:
Neuropathological findings in sporadic Creutzfeldt-Jakob disease (sCJD) brains are usually investigated using formalin-fixed and formic acid-treated brain tissues. Pathological evaluation is usually qualitative, but quantitative methods are being developed. Once brain tissues are fixed with formalin, they are limited for uses other than neuropathological analysis. It is highly desirable to be able to perform quantitative and biochemical analyses on formalin-fixed brains. Therefore, we developed Real-time quaking-induced conversion (RT-QuIC) assays to quantitate the seeding activity (SD_{50}) of sCJD brains.

Materials and Methods:
We used endpoint RT-QuIC assays to analyze 19 brain samples obtained from definite cases of 19 sCJD patients: 14 cases of MM1, 3 cases of MM2-thalamic form (MM2T), and 2 cases of MM2-cortical form (MM2C). Sporadic CJD was diagnosed according to Parchi's classification. The entire cortex was treated with formalin for 1 month. The samples were subsequently exposed to 99% formic acid for 1 hour. To examine prion distribution in different parts of the brain, we analysed prion seeding activity by using endpoint RT-QuIC assay in six different lobes (frontal, temporal, parietal, occipital, thalamus, and cerebellum) of MM1 (n = 2), MM2T (n = 2), and MM2C (n = 1) samples. We also compared the neuropathological findings with SD_{50} from formalin-fixed brain samples from MM1, MM2T, and MM2C patients. The assay was repeated at least two times, and Spearman–Kärber analysis was used to estimate a seeding dose (SD_{50}).

Results:

The SD_{50} values of formalin-fixed brain samples from fourteen MM1 cases, two MM2C cases, and two MM2T cases were $10^{7.77\pm0.57}$/g tissue, $10^{7.44\pm0.24}$/g tissue, and $10^{6.00\pm0.77}$/g tissue, respectively. The SD_{50} values decreased by $10^{2.04}$ after formalin fixation for 1 month. After combined formalin and formic-acid treatment, the SD_{50} value was reduced by approximately $10^{5.16}$ compared with the native brain in MM1 cases. Moreover, here, we initially compare the degree of vacuolation, PrP deposition, and SD_{50} in sCJD patients. We found that there was a relationship between SD_{50} and neuropathological findings in the cerebral cortex except for in the occipital lobe. Finally, we found that there was a difference between grey matter and white matter of SD_{50}. The smallest in the MM1 cases, at approximately 0.05, larger in the MM2T cases, at approximately 0.25, and the largest in the MM2C, at 0.44. After further treatment with formic acid, the difference between grey matter and white matter was the smallest still in the MM1 cases (approximately 0.1), and in MM2T and MM2C cases was 0.28 and 0.25, respectively.

Discussion:

In this study, we successfully measured prion-seeding activities in formalin-fixed brain samples and in formic acid-treated formalin-fixed brain sCJD patients (MM1, MM2T, MM2C) using RT-QuIC. Our study showed that the prion seeding activity reduces more than 100.000 times after treating with formic acid in formalin-fixed brain MM1sCJD patients. We assumed that an SD_{50} titer of 7–7.5 is the limiting point of transmission for humanized knock-in mice. From our research, we found that the formalin-fixed brain tissue of MM1, MM2C was infectious but MM2T was no infectious. All samples after combining the formalin and the formic acid treatment were no infectious. Besides, we had compared the degree of vacuolation, PrP deposition, and SD_{50} and in sCJD patients. There was a relationship between SD_{50} and neuropathological findings in the sCJD patients. In this research, we initially gave an accurate index of SD_{50} of white matter and grey matter when treated with formalin and formic acid in sCJD patients. We also found a slight difference between the prion seeding activity in the white matter and grey matter in sCJD patients following formalin and formic acid treatment.

With succeeding in quantifying prion seeding activities in formalin-fixed and/or formic acid-treated brains of sCJD patients. This lets us know the risk of transmission when medical workers handle highly infectious human samples. End-point RT-QuIC with formalin-fixed sCJD in the brain and many organs will be a useful tool in combination with neuropathological analysis to improve understanding of sCJD. Moreover, in the future, formalin RT-QuIC has become the most potent tool for the in vivo diagnosis of not only prion diseases but also synucleinopathies and tauopathies.