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学位論文名	Analysis of microdissected human neurons by a sensitive ELISA reveals a correlation between elevated intracellular concentrations of Aβ42 and Alzheimer's disease neuropathology （高感度ELISAを用いたヒト単離ニューロンの解析で明らかとなったAβ42の細胞内濃度上昇とアルツハイマー病の神経病理との関連性）
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論文内容の要旨

〔目　的(Purpose)〕

In Alzheimer's disease (AD), Purkinje neurons in the cerebellum are spared, while, for instance, pyramidal neurons in the hippocampus are neuropathologically affected. Several lines of evidence suggest that the pathogenesis could be induced by the concentration-dependent polymerization of the amyloidβ-peptide (Aβ) into extracellular oligomers. The role of intracellular Aβ is not fully investigated, but recent data indicate that also this pool could be of importance. Here isolation of Purkinje neurons from AD cases and controls was performed using laser capture microdissection (LCM) microscopy, and the low levels of intracellular Aβwas quantified using a novel and highly sensitive ELISA, in which the sensitivity was increased ten times compared to a conventional ELISA. Moreover, the present approach made it possible to calculate the intraneuronal concentrations of Aβ, which may correlate to the AD pathology. Thus, the aim of the present study is to quantify the intracellular levels of Aβ40 and Aβ42 and to investigate whether there is a correlation between Aβ levels and the neuropathological changes observed in AD.

〔方法 (Methods)〕

Brain samples : All human materials were obtained from the Huddinge Brain Bank at Karolinska Institutet Alzheimer Disease Research Center, and the study was approved by the local ethical committee at Karolinska Institutet. All AD brains met clinical criteria for definitive AD and neuropathological CERAD (Consortium to Establish a Registry for Alzheimer's Disease) criteria

ELISA: The sandwich ELISA system from Wako Chemicals GmbH was adopted as a conventional ELISA (Aβ40: Catalog No. 294-64701, Aβ42: Catalog No.292-64501). To enhance the sensitivity of the ELISA, the substrate was changed from the colorimetric substrate provided with the kit to the fluorescent substrate, Amplex UltraRed.

Preparation of samples for Aβ measurement in sections: The cerebellar cryosections (10 μm) adjacent to those for LCM were cut out from the slide glass with a sharp blade. Each cerebellar section on the membrane was collected into an eppendorf tube adding the RIPA buffer. Formic acid was added into the tube and incubated for 20 h. The final concentration of formic acid was 70%. Aliquots of each formic acid extract were neutralized in 1 M Trizma base, and five times concentrated RIPA buffer was added to the tube. The protein quantification was performed using FluoroProfile Protein Quantification Kit

LCM and preparation of neuronal samples: Using the Laser Microbeam System (PALM Microlaser), 500 Purkinje neurons per case were catapulted into an LPC-Microfuge tube cap. After addition of formic acid and 10 min sonication, the neuronal sample was incubated overnight to be dissolved at room temperature. An aliquot of each formic acid extract was neutralized 1 M Trizma base. The neutralized sample was mixed with five times concentrated RIPA to avoid the aggregation of Aβ.

〔成績(/Results)〕

ELISA development: It was found that it is necessary to reduce the influence of the sample buffer by further dilution (eight times) with RIPA buffer. Furthermore, since IHC data indicated that the intracellular Aβ40 and Aβ42 levels were low, an increased ELISA sensitivity was required. By using Amplex UltraRed instead of the colorimetric substrate included in the ELISA kit, the sensitivity of the ELISA was increased around ten times.

Aβ measurement in sections: I found an increase of Aβ42 in cerebellar sections from sporadic AD (sAD) cases compared to controls (1.2 and 0.037 fmol/μg protein, respectively), which is consistent with a previous report. Still, the levels were clearly lower than the Aβ42 levels in the hippocampal sections from sAD cases (40 fmol/μg protein, previously reported). The mean level of Aβ40 was higher in the sAD cases (0.59 fmol/μg protein) compared to controls (0.031 fmol/μg protein) but this was not statistically significant.
Intraneuronal concentration of Aβ: The intracellular Aβ40 level in Purkinje neurons was similar in the control and the AD group. On the other hand, significantly increased amounts of Aβ42 were detected in the sAD group, and the Aβ42/Aβ40 ratio was higher than in control cases. Based on the area of neurons captured by LCM and previously reported data, the concentration of intraneuronal Aβ was calculated. In sAD cases, the mean concentrations of Aβ42 in Purkinje and CA1 pyramidal neurons were around 200 nM and 3 μM, respectively. In control cases, the Aβ42 concentration in Purkinje and CA1 pyramidal neurons were 90 and 660 nM, respectively. The concentrations of Aβ40 in Purkinje and CA1 pyramidal neurons were 1 and 10 μM, respectively.

〔総　括(Conclusion)〕

It was reported in this study that the Aβ40 concentration in Purkinje neurons and CA1 pyramidal neurons were 1 and 10 μM, respectively. It may be of interest to note that these values are below the critical concentration for the polymerization of Aβ40 into fibrils, which has been suggested to be around 14 μM. It has been reported that 1 μM of Aβ42 is sufficient to support the growth of aggregates. Thus, Aβ42 in CA1 pyramidal neurons most likely forms aggregates or oligomers in pyramidal neurons, since the intracellular concentration is 3 μM. On the other hand, Aβ42 in Purkinje neurons was rather low level. Finally, It was suggested that high concentration of intracellular Aβ42 correlates with vulnerability to AD neuropathology.

論文審査の結果の要旨

βアミロイド（Aβ）仮説によると、細胞外Aβの重合体形成がアルツハイマー病における神経細胞死のトリガーであると考えられてきたが、細胞外Aβの蓄積部位と神経細胞死が起こる部位は必ずしも一致していない。一方、細胞内Aβがアルツハイマー病に重要な役割を果たすことが近年示唆されているものの詳細は分かっていない。本研究ではまず、高感度ELISA法を開発し、その手法を用いてヒト死後脳より単離した神経細胞内Aβ40及びAβ42の量を定量した。さらに、神経細胞の体積を測定することにより、海馬錐体神経細胞及びプルキンエ細胞でのAβ濃度を決定した。その結果、アルツハイマー病で脆弱性を示す海馬錐体神経細胞では、細胞内Aβ42濃度が多量体へ重合を開始すると予想される濃度以上に高まることを見出した。よって、本研究はアルツハイマー病における神経細胞脆弱性と細胞内Aβ濃度の関係に対して定量的考察を行ったことにより、博士（医学）の学位授与に値するものと認める。