

研究課題名

The localization of Amyloid beta in mice brain

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1) Introduction

Alzheimer's disease (AD) is the most common cause of dementia worldwide and a chronic, progressive neurodegenerative disorder. AD is characterized by early memory loss, followed by the gradual decline of other cognitive functions; impairment in visuospatial skills, complex cognition, language, emotion and personality (Jurgen et al., 2008). Although AD was first described in 1906 and since then extensive researches have been done, the exact cause of AD remains unclear and there's no cure and even effective treatment yet (Akiyama et al., 2000). More than 20 million people worldwide are suffered from AD (Brookmeyer et al., 2007). Furthermore, because of the increasing life expectancy of human population, the number of AD patients is predicted to double throughout the world on average every 5 to 6 years (Ziengler-Graham et al., 2008).

AD patients need care of nursing homes or institution and therefore the social and economic costs for AD patients are enormous. Furthermore, AD is one of the main causes of reduced life quality (Wimo et al., 2007). As a result, understanding AD aetiology and developing effective treatment for AD is critical problem for human beings.

As one of instruments to solve this problem, there are gene-targeted and transgenic mice. Although no mouse model can recapitulate AD pathology completely, this technology is invaluable to model various aspects of AD neuropathology (Wong et al., 2002). As a hallmark lesion of AD, beta-amyloid plaques and neurofibrillary tangles (NFT) are suggested. Amyloid-beta plaques contain the polypeptide amyloid-beta which is derived from amyloid precursor protein (APP). While neurofibrillary tangle is consist of hyperphosphorylated aggregates of the microtubule-associated protein tau (Selkoe, 2001). The amyloid hypothesis (Hardy and Allsop, 1991; Hardy and Selkoe, 2002) suggests the accumulation of amyloid-beta is the primary influence driving AD pathology and it is supposed that the accumulation of amyloid-beta results from an imbalance between amyloid-beta production and amyloid-beta clearance. However, the

memory and cognitive deficits correlated better with the loss of neuronal synaptic density and synapse number than the presence of beta-amyloid plaques or NFTs, it's still elusive the interaction between amyloid-beta and tau and their effect on synaptic function (Ditte Z et al., 2010).

The overall aim of this study is to acquire the technique of Immunohistochemistry by using 3xTg-AD mice and testing the localization of the accumulation of beta amyloid plaques in mice brain.

2) Materials and methods

Unless stated, all reagents were supplied by Sigma-Aldrich, UK. This study used non-transgenic mice and triple - transgenic mice (3xTg-AD) harboring three mutant genes, amyloid-beta precursor proteins, penisenilin1 and tau (Odd et al., 2003). Following cervical dislocation and decapitation the brain was rapidly removed. Then the brain was placed on its posterior end on silver foil on dry ice until completely frozen. Brains were stored at -80°C until subsequent cryostat processing. Before sectioning, whole brains were cut in half. Brains were attached to the cryostat chuck using OCT, and orientated so the frontal lobes were positioned anteriorly. Coronal brain sections(30 μ m thick) were taken, placed in a well with antifreeze and stored at -20°C until stained.

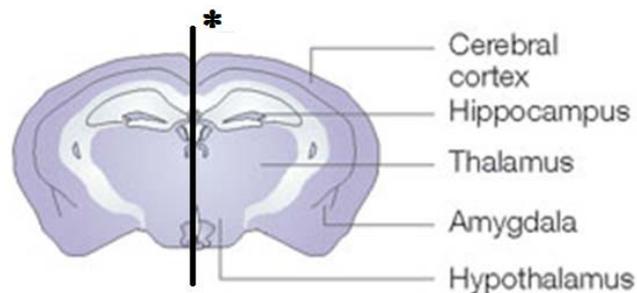


Figure.2 Coronal brain section

In this study, half brain was used for immunohistchemistry.

For amyloid-beta staining, single staining was performed. After washing by PBS, brain sections were treated with 0.3% H₂O₂ solution to block the endogenous peroxidase. Non-specific site were blocked by 10% normal horse sera (NHS) in PBS/0.3% triton prior to addition of the primary antibodies diluted 1:3000 by using 1%NHS/PBS/0.3%triton. Then these samples were incubated overnight at 4°C. Next day, after washing by PBS, the secondary antibody, horse biotinylated anti-mouse (Vector Laboratories, Burlingame, USA) diluted 1:1000 in1% NHS /PBS/0.3%triton

were applied for 2 hours in room temperature. These samples were visualized using the ABC method with Vectastain Kit (Vector Laboratories) and 50 mg/mL DAB as chromogen providing brown color (10 min exposure for samples). After these processes, each sample was mounted to a gelatin coated slide.

3) Result

3-1) non-transgenic mice



Figure 3.1 the accumulation of amyloid-beta in non-transgenic mice

A) Whole brain

B) amygdala

C) hippocampus

There's no plaque in these samples.

3-2) transgenic mice



Figure 3.2 the accumulation of amyloid-beta in transgenic mice

A) Whole brain

B) amygdala

C) hippocampus

Amyloid-beta deposits in amygdala and hippocampus.

3-3) 2 months old transgenic mice

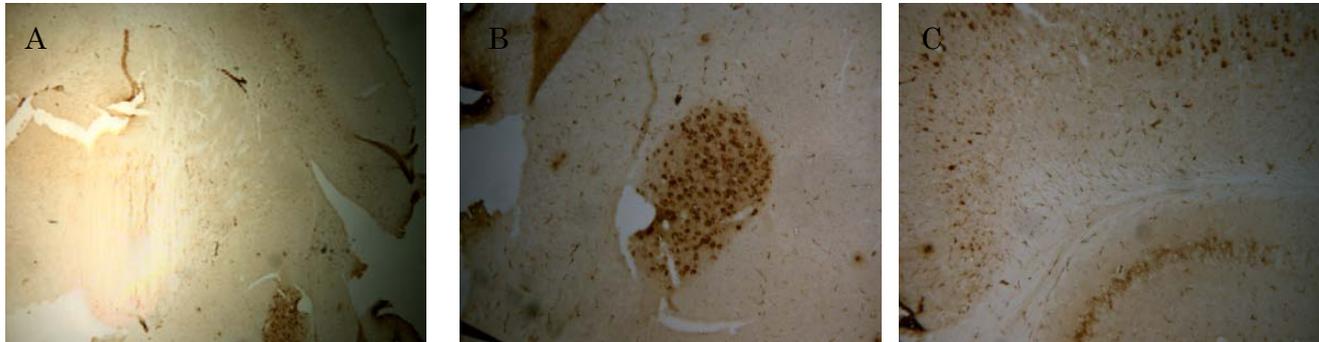


Figure 3.3 the accumulation of amyloid beta in 2 months old transgenic mice
A) Whole brain B) amygdala C) hippocampus and cortex
There're some plaques in amygdala, hippocampus and cortex.

3-4) 12 months old transgenic mice

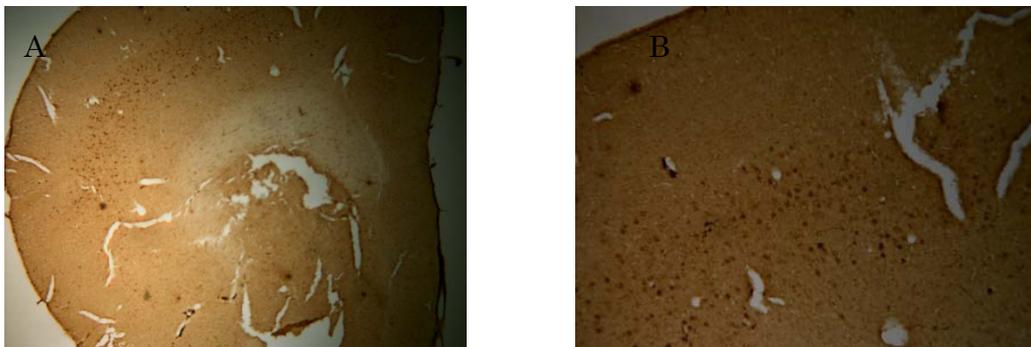


Figure 3.4 the accumulation of amyloid beta in 12 months old transgenic mice
A) Whole brain B) cortex
There're plaques in cortex.

4) Discussion

As previously published, it's well known that amyloid-beta peptide is extracellularly deposited in plaques predominantly in the amygdala, the hippocampus, and the neocortex of affected individuals (Van Gassen G, 2003). Therefore the results of 3-1) and 3-2) were considered proper, although why amyloid plaques deposit at these specific sites was unclear. The sample of non-transgenic mice didn't seem to have plaques, while the sample of 3xTg mice had apparently amyloid-beta plaques.

However, the results of 3-3) and 3-4) didn't fit the property of AD which is chronic disease. In these results, the samples of 2 months mice had more apparent plaques than the samples of 12 months mice had. For this reason, it's thought that the treatment of immunohistochemistry may have included some mistakes. To prevent this uncertainty, the accuracy of my research technique is needed.

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