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Effects of an amyloid-beta 1-42 oligomers antibody screened from a phage display library in APP/PS1 transgenic mice

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Abstract

We screened anti-A β 1-42 antibodies from a human Alzheimer's disease (AD) specific single chain variable fragment (scFv) phage display library and assessed their effects in APP/PS1 transgenic mice. Reverse transcription-PCR was used to construct the scFv phage display library, and screening identified 11A5 as an anti-A β 1-42 antibody. We mixed 11A5 and the monoclonal antibody 6E10 with A β 1-42 and administered the mixture to Sprague-Dawley rats via intracerebroventricular injection. After 30 days, rats injected with the antibody/ A β 1-42 mixture and those injected with A β 1-42 alone were tested on the Morris water maze. We also injected 11A5 and 6E10 into APP/PS1 transgenic mice and assessed the concentrations of A β in brain and

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Competing interests

The authors declare no competing interests.

Author contributions

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All relevant data are within the paper.

peripheral blood by ELISA at 1-month intervals for 3 months. Finally we evaluated behavior changes in the Morris water maze. Rats injected with A β 1-42 and mixed antibodies showed better performance in the Morris water maze than did rats injected with A β 1-42 alone. In APP/PS1 transgenic mice, A β concentration was lower in the brains of the antibody-treated group than in the control group, but higher in the peripheral blood. The antibody-treated mice also exhibited improved behavioral performance in the Morris water maze. In conclusion, anti-A β 1-42 antibodies (11A5) screened from the human scFv antibody phage display library promoted the efflux or clearance of A β 1-42 and effectively decreased the cerebral A β burden in an AD mouse model.

Keywords

Alzheimer's disease; Phage display library; Anti-A β antibodies; APP/PS1; transgenic mice

1. Introduction

Alzheimer's disease (AD), a neurodegenerative disease, is characterized by memory and cognition impairment. Amyloid- β peptide (A β) deposition triggers a series of extracellular events and processing changes that eventually lead to dementia, a key component in the pathogenesis of AD (Hardy and Selkoe, 2002). Therefore, inhibiting the production of A β is considered a potential therapeutic strategy in treating AD. As an extracellular protein, A β deposits aggregate in the associative cortices and limbic system. A β is derived from the β -amyloid precursor protein (β -APP), which is a larger transmembrane protein (Buss et al., 2012). A β is generated when APP interacts with the APP cleavage enzyme β -secretase (BACE1) in the endoplasmic reticulum, Golgi, cell surface and/or endocytic compartments, where BACE1 cleaves the N-terminal domain of APP (Birmingham and Frantz, 2002; Haass and Selkoe, 2007; Hock et al., 2003; Schenk, 2002; Selkoe, 2006). The abridged protein fragment (C99) contains the transmembrane domain, which interacts with γ -secretase (including presenilin-1, PS1) to release A β from the membrane (Selkoe, 2006).

Recent AD studies have focused on soluble A β (Haass and Selkoe, 2007). Schenk first used synthetic A β 42 to immunize the platelet-derived growth factor B promoter driving human amyloid precursor protein V717F (PDAPP) transgenic mice, determining that A β 42 reduced the extent and progression of AD pathology (Schenk, 2002). Since then, significant progress has been made in designing an appropriate human vaccine using active A β immunotherapy. However, in a phase II clinical trial, approximately 6% of patients suffered from meningoencephalitis, which led to a discontinuation of the application of A β (Birmingham and Frantz, 2002; Hock et al., 2003; Marks et al., 1992). Thus, a human-origin antibody against A β would be more promising for AD research and clinical therapy.

Phage display library technology (Petrenko and Vodyanoy, 2003; Szardenings, 2003; Willats, 2002) is a relatively simple and quick method of constructing effectively designed synthetic antibody libraries and obtaining considerable amounts of humanized antibodies (Keller et al., 2015).

Our study utilized a human single chain variable fragment (scFv) phage antibody library to isolate a single-chain antibody that specifically recognized A β 1-42 oligomers. Such

antibodies may prevent or reverse the deposition of A β in central nervous system; subsequently reducing associated behavioral deficits in AD mouse models.

2. Results

2.1. Screening of single-chain fragment variable antibodies

The total RNA was isolated from the lymphocytes and cDNA was prepared. The VH and VL bands amplified by RT-PCR were approximately 360 bp and 340 bp, respectively (Fig. 1A and B). The VH and VL DNA sequences were made continuously into an scFv using a pair of linker DNA sequences and special primers (all of the primer sequences were designed from Gene Bank). The scFv band was approximately 750 bp (Fig. 1C). The scFv fragment was inserted into the pCAN-TAB5E vector and amplified. The product was electroporated into *E. coli* TG1 and rescued by the M13K07 helper phage. Random colonies were selected from an SOBAG agar plate and plasmid DNA was separated (Fig. 1D).

Recombinant phages specific for A β 1-42 oligomers were enriched after 4 rounds of bio-panning and the antigen-positive clones were selected from the enriched clones by phage ELISA. The phage ELISA showed an increased trend in the every round of panning (Fig. 2). In the first round, the total output phage was 1.24×10^4 . The phage-containing supernatant was then amplified by infecting competent bacteria, titering, and pooling for the next round of screening. Subsequently, an enrichment of approximately 6.23×10^7 fold was reached.

2.2. Positive clones are enriched after four rounds of panning

After the final round of bio-panning, we choose the highest activity antibody of the positive clones-11A5 to display specific binding to A β 1-42 oligomers. The CDR regions in VH and VL amino acids were determined by using KABAT and Chothia numbering (Fig. 3 A–C). Western blotting was used to demonstrate the specific binding of antibodies to A β 1-42 oligomers. 11A5 could specifically bind to A β 1-42 oligomers and displayed a molecular weight of approximately 34 kD (A β 1-42 tetramer) (Fig. 4).

2.3. Anti-A β 1-42 antibodies could neutralize A β 1-42 peptide in vivo

We used the Morris water maze to assess behavioral changes of SD rats after 30 days. The escape latency (the time needed to reach the hidden platform) was compared among the groups. The results demonstrated that the time required was much shorter for the A β 1-42 antibody treatment groups than for the A β 1-42 group (Fig. 5A, $P < 0.01$). The results of the spatial probe test revealed that the A β 1-42 antibody treatment groups spent much more time in the target quadrant than the A β 1-42 group (Fig. 5B, $P < 0.01$). A β 1-42 antibody treatment led to a significant increase of the number of times in platform location compared to A β 1-42 group (Fig. 5C, $P < 0.01$). However, no significant differences were observed between the groups receiving A β 1-42 mixed with 11A5 or A β 1-42 mixed with 6E10 and the sham group ($P > 0.05$). It was seemed the rats of A β 1-42 mixed with antibodies groups showed mild reduction in learning and memory at day 30 after treatments compared with sham group. This suggests that anti-A β 1-42 antibodies (11A5, 6E10) could combine with and deactivate the A β 1-42 peptide, thereby decreasing its neurotoxic effects on SD rats.

2.4. Behavioral tests of APP/PS1 transgenic mice

The PCR results identifying double transgenic mice are shown in Fig. 6A and B. Double positive gene expression mice were chosen for this study. In the Morris water maze test, the escape latency and spatial probe test were compared among the groups at 1-month intervals for 3 months. The escape latency results demonstrated that the time spent by the antibody treatment groups was much shorter than the time spent by the control group (Fig. 7A–C, $P<0.01$). The spatial probe test data revealed that the antibody treatment groups spent much longer times in the target sector than the control group (Fig. 7D, $P<0.01$. Fig. 7E and F, $P<0.05$). The antibody treatment groups also located the platform significantly more often than the control group (Fig. 7G–I, $P<0.05$). However, the data showed no significant differences between the 11A5 and 6E10 treatment groups ($P>0.05$). Therefore, the results indicated a significant improvement in abilities of learning and memory after 11A5 and 6E10 injections.

2.5. Detection the A β concentrations in brain and peripheral blood

The concentrations of A β were tested by ELISA (Fig. 8A and B). The concentrations of A β in brains were lower while the level in peripheral blood were higher in the treatment groups than control group and A β concentrations were lowest in brains but highest in peripheral blood at the first month in the treatment groups ($P<0.01$), which indicated that 11A5 could combine with A β and might initiate A β efflux or clearance from the brain to the peripheral blood and the effects was strongest at the first month.

3. Discussion

In our study, we successfully screened anti-A β 1-42 oligomers antibodies (11A5) from a human scFv antibodies phage display library that we designed and confirmed the ability of 11A5 to combine with A β and make it deactivate in vitro through the tests of A β 1-42+antibodies mixtures intracerebroventricular injections on SD rats. We also confirmed 11A5 might initiate A β efflux or clearance from the brain through the tests of antibodies treatments on APP/PS1 transgenic mice. Furthermore, anti-A β 1-42 antibodies (11A5) therapy was effective in improving behavioral abilities of APP/PS1 transgenic mice. Following treatment, anti-A β 1-42 antibody (11A5) was proved to combine with A β and enhance the efflux or clearance of A β from the brain. To the best of our knowledge, this study is more clearly and deeply to explore the therapeutic effects of humanized anti-A β 1-42 oligomers antibodies in AD mouse models.

Over the past twenty years, monoclonal antibodies have become an important class of pharmaceutical biotechnology products and research tools in chemistry and the life sciences (Sliwkowski and Mellman, 2013). The advent and development of phage display library technologies enabled efficient isolation of fully human antibodies from large combinatorial repertoires (Smith, 1985; Winter et al., 1994).

In our study, we constructed a human scFv antibody phage display library and obtained a single-chain antibody that specifically recognized A β 1-42 oligomers. The scFv library was constructed using the peripheral blood of 20 AD patients, which improved the variety, library productivity, and screening rate of positive clones. 11A5 is a conformation-

dependent human monoclonal antibody that it can be obtained under more simple and economic condition. We have demonstrated it has strong biological activities similar as the commercial antibody 6E10. The monoclonal antibody 6E10 is the mouse parent antibody, and many disadvantages of these antibodies have been highlighted: on the one hand, they cannot effectively activate system of complements and Fc receptors in the human body. On the other hand, they can be identified by the body's immune system to develop the human antigen mouse antibodies. Furthermore, they will be removed in the human body. 11A5 is a human monoclonal antibody that can be obtained under more simple and economic condition (in our lab). We have demonstrated that it has strong biological activities similar with the commercial antibody 6E10. As a humanized antibody, 11A5 may reduce the severity of immune responses in human, which is presenting superior to 6E10. Thereby presenting a possible application as a therapy in clinical research.

A β has an important role in AD pathology, as its extracellular deposits constitute senile plaques that induce the most common pathologic changes (Allsop et al., 1983; Glenner and Wong, 2012). The A β 1-42 oligomers might have higher antigenicity than other A β 1-42 forms, as been previously reported (Yuan et al., 2013). Many studies are committed to developing synthetic antibodies against A β oligomers to prevent neuron degradation or apoptosis; the efforts are ultimately aimed at improving central nervous system regeneration and prevent lasting memory loss (Klyubin et al., 2005; Zameer et al., 2008). The first anti-A β immunotherapy approach for AD, which was introduced by Schenk in 1999. Despite some disappointments, the researches of anti-A β immunotherapy are never stopped. Several more effective active or passive A β -based vaccines have been developed. Including the usage of IVIg that contain naturally occurring anti-A β antibodies. (Relkin et al., 2009; Weksler et al., 2009) Recent studies focus on using conformation-specific antibodies 49 which can recognize soluble oligomers from different amyloids, including islet amyloid polypeptide (IAPP), lysozyme, synuclein, polyglutamine, prion protein, and insulin. These anti oligomer antibodies that bind to amyloid oligomers (Kayed et al., 2003) specifically when injected intrathecally into the TgCRND8 AD mouse model (Chauhan, 2007).

Because specific antibodies against A β constructs are present higher levels in the plasma and serum from AD patients than healthy elderly people (Silacci et al., 2005), we developed a phage library from the lymphocytes of AD patients to screen for human anti-A β antibodies. We made preparation for A β 1-42 oligomers for antibody selection and obtained specific antibodies.

In our study, we made intracerebroventricular injection on SD rats A β 1-42 or A β 1-42 mixed with antibodies, the changes of behavior of A β 1-42 mixed with antibodies groups were no statistical differences compared with sham group (only injection of normal saline), but the changes were significant differences compared with A β 1-42 group, which demonstrated anti-A β antibodies (11A5) could combine with and deactivate A β 1-42. In APP/PS1 transgenic mice tests, we intraperitoneal injected either anti-A β antibodies (11A5) or commercialized antibodies (6E10) into APP/PS1 mice at 4 months of age. The injections were repeated every two weeks. We had observed a series of decrements of A β levels in brain and increments of A β levels peripheral in blood during 3 months, which demonstrated that the anti-A β antibodies could reduce A β levels by efflux or clearance from brain.

The neurodegenerative process of AD begins with axonal and synaptic damage and is accompanied by the gradual aggregation of toxic A β in the intracellular and extracellular space. A β aggregation is the consequence of an altered balance between protein synthesis, accumulation rate, and clearance. Two major mechanisms for the antibody-mediated clearance of A β have been suggested, including the sequestration of A β from the central nervous system into the periphery ("peripheral sink") (Bard et al., 2000) and the entry of anti-A β antibodies into the central nervous system and clearance of antigen-antibody complexes. That is to say, once the anti-A β antibodies are supplied via intraperitoneal injection into the APP/PS1 mice, a rapid increase in the plasma A β levels will be observed (DeMattos et al., 2001). The circulating anti-A β antibodies bind to plasma A β , reducing the circulating A β levels. This reduction initiates the migration of A β from the brain by mass action transfer across the blood brain barrier to the vasculature, which is consequently termed the peripheral sink. In addition, with the equilibrium of A β between the brain and blood has been broken, the entry of anti-A β antibodies into the central nervous system has been speeded (Lafaye et al., 2009). In our study, we observed decreases concentrations of A β in brain and corresponding increases concentrations of A β in peripheral blood after treatment with the anti-A β antibodies. It indicated that 11A5 could reduce the A β load by promoting the efflux or clearance of A β from the brain to peripheral blood. The effects of anti-A β antibody combined with A β might initiate it. The deposition of A β fragments in senile plaques leads to neurodegeneration and neuronal death, and though the present study focused on anti-A β therapy as a means to reduce A β deposition in the brain, it is unlikely that only A β alone brought about the total decline in cognitive abilities in the AD model mice. Indeed, the Morris water maze test revealed that cognitive improvement was not as significant in the context of the escape trial. Therefore, if more mechanisms of neurodegeneration are identified and regulated simultaneously, cognitive recovery might be more substantial.

4. Conclusions

We successfully screened an anti-A β 1-42 oligomers antibody (11A5) from a human scFv antibodies phage display library that we developed in-house. We also demonstrated the ability of 11A5 to combine with and deactivate A β 1-42. Moreover, therapy with 11A5 effectively improved the behavioral abilities of APP/PS1 transgenic mice. Anti-A β 1-42 antibody (11A5) could not only combine with and deactivate the A β 1-42 peptide directly but also initiate A β efflux or clearance from the brain. These results suggest that humanized anti-A β antibodies therapy may have a positive effect in treating AD.

5. Experimental procedures

5.1. Ethics Statement

All patients provided written informed consent to participate in this study. Animals were treated according to the standards of Animal Care and Use Committee of Zhengzhou University. All procedures in our study were approved by the Ethics Committee of Zhengzhou University.

5.2. Construction and cloning of phage antibody library

We collected peripheral venous blood from 20 AD patients in heparin-containing tubes. The blood was used within a few hours of collection. We diluted 10 mL of blood with an equal volume of PBS and carefully overlay 10 mL of the diluted blood with 10 mL of Lymphocyte Separation Medium (Human) (Solarbio, Beijing, China) and centrifuged the tubes at 1500g for 25 min at room temperature (RT). Removed most of the upper layer and collected the buffy coat between the two layers carefully, just the layer of cells, into 2 mL Ep tubes. Suspended the cells in PBS and centrifuged the tubes at 13,000g for 3 min, removed the supernatant and repeated the process for three times. The sediments in the tube were lymphocytes.

Total RNA was extracted from lymphocytes with Trizol™ kit (CWBIO, Beijing, China) according to the manufacturer's instructions and was reverse transcribed to first-strand cDNA in DEPC-treated water using 1 µL random hexamer primer (ThermoScript™ RT-PCR System, Invitrogen, Grand Island, NY, USA), 2 µL of 10 mM dNTP-Mix (Invitrogen), 1 µL 0.1 M DTT (Invitrogen), 1 µL RNAOUT™ (Invitrogen) and 1 µL ThermoScript™ RT (Invitrogen) in a final volume of 20 µL. Samples were denatured by incubating at 65 °C for 5 min, followed by 50 min at 50 °C. The cDNA obtained was stored at – 20 °C.

cDNAs coding for immunoglobulin heavy chains (VH) and light chains (VL) were amplified with different primers (synthesized by Sangon Biotech, Shanghai, China) and were used to amplify a majority of the known human antibody sequences via reverse transcription- polymerase chain reaction (RT-PCR) (Brezinschek et al., 1995, 1998; Huang and Stollar, 1991). The 20 µL reaction volumes contained 2 µL of cDNA, 1 µL of each primer, 1 µL 10 mM dNTP-Mix, 5 µL 10 × PCR buffer and 0.2 µL Taq DNA polymerase (Invitrogen). The cycling parameters were 94 °C for 30 s (denaturation), 55 °C for 30 s (annealing) and 72 °C for 1 min (extension) for 31 cycles. The purified VH and VL DNA were made into an scFv fragment through a pair of linker DNA fragments (Sangon Biotech) using T4DNA Ligase (CWBIO) at 22 °C for 1 h. The fragments were then amplified with special primers by Taq DNA polymerase (Invitrogen) at 94 °C for 35 s (denaturation), 57 °C for 30 s (annealing) and 72 °C for 1 min (extension) for 35 cycles. All products were confirmed by 0.8% agarose gel electrophoresis. The amplified VH and VL fragments were approximately 340 bp and 360 bp in size respectively, and the size of scFv fragments was approximately 750 bp.

The scFv fragments and the pCANTAB-5E vector (Bio-viewshine, Beijing, China) were digested with Sfi I and Not I restriction enzymes (New England Biolabs, NEB, Beverly, MA, USA) overnight at 37 °C. Then, they were ligated at a 3:1 (insert: vector) ratio for 5 min at 25 °C with Quick T4 DNA ligase (NEB) and electroporated into competent E. coli TG1 cells (NEB). The products were rescued by the M13K07 helper phage (Bio-viewshine). E. coli TG1 cells were electroporated using a MicroPulser Electroporation Apparatus (Bio-Rad, Richmond, CA, USA) and 0.1 cm electroporation cuvettes. Following recovery, the cells were plated in ampicillin-containing media and grown overnight at 37 °C. The cells were resuspended in 2 × YT-AK medium. The cultures were incubated overnight on a rocking platform at 250 rpm at 37 °C. And then were centrifuged at 13,000g for 10 min. The

supernatant containing the recombinant phage was stored at 4 °C for the next step of bio-panning.

5.3. Antibodies from the scFv phage antibody library were selected based on their specificity for A β 1-42 oligomers

Preparation of A β 1-42 oligomers: 1 mg A β 1-42 peptide (Sangon Biotech) in lyophilized form was dissolved in 500 mL 1, 1, 3, 3, 3-hexafluoro-2-isopropanol (HFIP) and incubated for 1 h at 25 °C and then was aliquoted into small aliquot and dried using a speed-vac. The dry peptide was stored at -20 °C for the next step. The dry peptide was dissolved in borate buffered saline (50 mM BBS/PBS) and reacted with 5 mM glutaraldehyde at 37 °C overnight to generate stable oligomers by controlled polymerization. The solution was neutralized with Tris buffer then dialysed against deionized distilled water overnight and lyophilized. It could be re-solubilized in deionized distilled water and diluted in PBS until required (Goni et al., 2010, 2013).

50 Colony units of scFv phage antibody library were placed in 96-well plates coated with prepared A β 1-42 oligomers, which was pre-incubated with a bovine serum albumin-blocked polyvinylidene difluoride membrane to prevent non-specific binding. After 60 min at 37 °C, the membrane was washed by shaking 5, 10, 10 and 10 times with PBS, PBST for rounds 1, 2, 3 and 4 in several. After incubating at 37 °C overnight, phages were collected by centrifugation and resuspended in 3 mL of PBS containing 1% bovine serum albumin. The input and output phages were titrated onto SOB-ampicillin-tetracycline agar plates to calculate the enrichment ratios. The eluted phages (100 μ L) were selected and enlarged by infecting E. coli HB2151 cells (NEB) with M13K07 helper phage after each round to coat a SOBAG tablet, which was then incubated at 30 °C for more than 20 h. The samples were placed in plates coated with A β 1-42 samples, with M13K07 as a negative control and 2 $\times$ YT as a blank control. The samples were blocked with 3% bovine serum albumin and washed 3 times with PBST. Next, 100 μ L horseradish peroxidase-conjugated anti-M13 antibody (HRP/anti-M13, Amersham Biosciences, Bath, UK) was added (1:5000 in PBS containing 2% (v/v) bovine serum albumin), and the samples were incubated for 1 h at 37 °C. Following 6 washes with PBST, the clones were treated with tetramethylbenzidine substrate (TMB) and the reaction was terminated with 50 μ L of 2 mol/L H₂SO₄. Analysis of the specificity of scFv antibodies to A β 1-42 oligomers by Western blot according to Western blot assay (Cloutier et al., 2000). We used 11A5 (1:100) as the primary antibody and the secondary antibody with HRP/anti E-tag (1:10000; Abcam, Cambridge, UK).

Enzyme-linked immunosorbent assay (ELISA) was performed to screen for positive clones (11A5). Clones were considered positive when the A_{450 nm} was more than 3 times the signal observed in wells with M13K07 alone. The phage-mid derived from clone 11A5 was used for DNA sequencing.

5.4. Animals

20 male Sprague-Dawley (SD) rats weighing 200–300 g were purchased from the Model Animal Research Center of Zhengzhou University in China (Zhengzhou, China).

60 APP/PS1 (APP^{swe} × PS1^{dE9}) double-transgenic mice in the C57BL/6J genetic background and weighing 20–25 g were purchased from the Model Animal Research Center of Nanjing University in China (Nanjing, China). These mice display immature and progressive plaques along with neuritic pathology accompanied by high levels of A β 1-42 beginning at 4 months of age (Wang et al., 2009).

Animals were housed five to a cage and maintained on a 12-h light/dark cycle (lights on at 8 a.m.). Food and water were freely available. Every precaution was taken to limit the numbers of animals used and to minimize their suffering.

5.5. Combined injection of anti-A β 1-42 antibodies and A β peptide into SD rats

Synthetic A β 1-42 (Sangon Biotech) (1 mg) was dissolved in sterile distilled water at a concentration of 2.5 nmol/ μ L and incubated at 37 °C for 24 h. Anti-A β 1-42 antibodies 11A5 or commercialized antibodies 6E10 were added to the A β 1-42 solution (at a ratio of 4:1) in a 200 μ L reaction volume (A β 1-42 alone as control). The samples were incubated at 37 °C for 3 h and centrifuged for 5 min at 13,000g, the supernatants were removed, and the samples were incubated for an additional 1 h at 37 °C. SD rats were divided into 4 groups ($n = 5$ /group) and received intracerebroventricular injection (i.c.v.) of 25 nmol/rat of A β 1-42 alone, or A β 1-42 mixed with 11A5 (25 nmol/rat, the content of 11A5 was 20 nmol/rat), or A β 1-42 mixed with 6E10 (25 nmol/rat, the content of 6E10 was 20 nmol/rat). An equivalent volume of normal saline was administered to the sham group (Bergin and Liu, 2010). After 30 days, behavioral changes in the 4 groups were assessed using the Morris water maze (Klapdor and van der staay, 1996; Vorhees and Williams, 2006).

5.6. Using anti-A β 1-42 antibodies to treat APP/PS1 transgenic mice

We used PCR analysis of tail DNA to confirm the genotypes of APP/PS1 transgenic mice. Tail biopsies were acquired and genomic DNA was prepared using a TIANcombi DNA Lyse&Det PCR Kit (TIANGEN BIOTECH, Beijing, China). The oligonucleotides were designed to amplify a 344 bp fragment of the APP gene and a 600 bp fragment of the PS1 gene, including the mutation site (Borchelt et al., 1997; Zhang et al., 2011). The primers for APP, PS1 and GADPH (Sangon Biotech) were as follows: APP, 5'GACTGACCACTCGACCAGGTTC TG 3' (sense) and 5'CTTGTAACCTTGGATTCTCATATCCG 3' (anti-sense); PS1, 5'AA TAGAGAACGGAGGAGCA 3' (sense) and 5'GCCATGAGGGCACTAATCAT 3' (antisense); control GADPH, 5'GCUGAUCCAUGACAACUGGTT 3' (sense) and 5'AAAGCC-GUCAUCCAUGACCTT 3' (antisense). The tail tissues were homogenized with a grinding pestle and were prepared according to the manufacturer's instructions. The reaction cocktail included 10 μ L of 2 × Det PCR MasterMix, 0.5 μ L of each primer, 1.0 μ L of sample DNA, and DEPC water to a final volume of 20 μ L. The cycling conditions were 94 °C for 3 min, 94 °C for 30 s (denaturation), 55–63 °C for 30 s (annealing) and 72 °C for 1 min (extension) for 35 cycles, followed by 72 °C for 5 min. The products were confirmed by 0.8% agarose gel electrophoresis. Numbers of the mice with double-positive gene expression was 45.

The mice with double-positive gene expression were chosen and randomly divided into 3 groups: control, 11A5 (0.1 nmol/ μ L), and commercialized antibodies 6E10 (0.1 nmol/ μ L) (Santa Cruz Biotech) ($n = 5$ for each group and time point). There were no significant intergroup differences in the animals' body weights. The treatment groups received a total of six 200 μ L intraperitoneal (i.p.) of either 11A5 or 6E10 every 2 weeks (Bard et al., 2012). Control group mice were given an equivalent volume of PBS. At 1, 2, 3 month, the Morris water maze was used to evaluate changes in behavior. Mice were anesthetized with 10% chloral hydrate (400 mg/kg) and sacrificed. The brains and peripheral blood were carefully collected to assess the secretions of A β by ELISA.

The Morris water maze was used to detect behavioral changes in animals (Francis et al., 1995; Klapdor and van der staay, 1996). Cylindrical pools (150 cm in diameter, 50 cm high for rats; 120 cm in diameter, 50 cm high for mice) were filled with 24 °C water (white-dyed water for APP/PS1 transgenic mice). An escape platform (12 cm in diameter) was placed 1–2 cm (2 cm for rats; 1 cm for mice) below the water surface and was fixed in the middle of the southeast quadrant of the pool. On each of 4 continuous days, animals were given 4 swimming attempts. For each attempt, the animals were released from a starting point chosen from the other quadrants, and the time spent to reach the platform (escape latency) was recorded. If animals could not find the platform within 90 s, they were placed on the platform for 30 s. The average time for the 4 swimming attempts was as the latency score for each animal. The averages were calculated for every group each day. After the last attempt on the final day, an additional attempt was performed with the platform removed (probe test) to examine spatial reference memory. When the platform was removed from the pool, the animals were allowed to swim freely for 120 s. The time that the animals spent swimming in the southeast quadrant (where the platform had been located) and the number of crossings over a point where the platform had been were recorded. After each trial, animals were dried and returned to their home cages.

The concentrations of A β in brains and peripheral blood were measured using a Beta-Amyloid Total A β ELISA Kit (Covance, Princeton, NJ, USA) according to the manufacturer's instructions. Each sample was assayed in duplicate at appropriate dilutions so that the relative luminescent units fell within the range of standard curves. Titers were recorded at an optical density of 450 nm. Anti-APP, anti-BACE1 and anti-PS1 antibodies were purchased from Sigma-Aldrich Co. LLC (St. Louis, Missouri, USA) for the Western blot. The hippocampi in each group were kept on ice for immediate homogenization in lysis buffer added with protease inhibitor. Samples were separated in SDS-PAGE gel and transferred to PVDF membrane. After blocked with 5% TBST, membranes were incubated with the above primary antibodies over-night in 4 °C and followed to the incubation with secondary antibody of a horseradish peroxidase-conjugated goat-anti-mouse IgG (1:5000, Sigma) for 1 h at room temperature. Membrane were washed for 5 min $\times$ 3 times in TBST between the incubations. Protein was detected by conducting reaction with Chemiluminescent Substrate (Invitrogen), exposing and developing the film.

5.7. Statistical analysis

All experimental data were statistically analyzed using the SPSS version 13.0 software. The results were presented as the means $\pm$ SD. One-way ANOVA was used to analyze the performance in the probe trial protocol; the differences in A β concentrations among 11A5, 6E10 treatment groups and control group. Bonferroni correction was used for the two-group comparisons. Repeated measures ANOVA followed by the least significant difference (LSD) test was applied to compare the escape latencies among groups. $P < 0.05$ was considered statistically significant.

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Fig. 1. Agarose gel electrophoresis showing PCR amplification of human antibody heavy chains (A, 360 bp), light chains (B, 340 bp) and scFv fragments (C, 750 bp). Recombinant clones of scFv fragments were amplified from the library and identified by PCR (D, 750 bp).

Fig. 2.

Bio-panning of the antigen-positive clones by ELISA. After the fourth round of bio-panning, antibodies were assessed by ELISA against A β 1-42 oligomers. Clones were considered positive according to the following criteria: absorbance A450 nm >0.5, and an A450 nm (positive control) to A450 nm (negative control) ratio >3.

Fig. 3. DNA sequences analysis. A and B: VH and VL chains' sequencing. C and D: DNA sequences (C: heavy chains D: light chains;) analysis for anti-Aβ1-42 antibodies (11A5). The CDRs for both the chains were obtained using KABAT numbering and the CDR sequences were shown in yellow highlight and the others were FR sequences that were shown in blue highlight.

Fig. 4. Western blot analysis of single-chain antibody 11A5 binding to A β 1-42 oligomers. M: marker; A: 11A5 specific binding to A β 1-42 oligomers. B: BSA (a bland control).

Fig. 5.

Changes of behavior after Aβ1-42, Aβ1-42 mixed with antibodies injections on SD rats were evaluated by Morris water maze. Aβ1-42: injection with only Aβ1-42; Aβ1-42+11A5: injection with Aβ1-42 and 11A5; Aβ1-42+6E10: injection with Aβ1-42 and 6E10; Sham: injection with normal saline. A: escape latency (Latency to find the platform in the Morris Water Maze test): rats receiving Aβ1-42 exhibited the worst performance than the other three groups. Rats receiving Aβ1-42 mixed with antibodies showed decreased the latency to find the platform (improved learning) compared with the Aβ1-42 group. * $P < 0.01$ vs. the Aβ1-42 group. B: spatial probe test (Quantification of time spent in the target zone during the probe test): rats receiving Aβ1-42 spent less time in the correct quadrant than the other three groups. Rats receiving Aβ1-42 mixed with antibodies spent much longer time in the target zone than did the Aβ1-42 group. * $P < 0.01$ vs. the Aβ1-42 group C: the number of times the platform: Aβ1-42 groups had the lest crossings of the annulus than the other three groups. * $P < 0.01$ vs. the Aβ1-42 group. Aβ1-42 mixed with antibodies groups showed slight difference with sham group. $n = 5/\text{group}$.

Fig. 6. PCR-genotyping to identify APP/PS1 transgenic mice from DNA acquired via tail biopsies. M: Marker; +: Positive control; -: Negative control; APP: 344 bp, PS1: 600 bp.

Fig. 7.

Results of the Morris water maze test of APP/PS1 transgenic mice. A, B, and C: escape latencies (Latency to find the platform in the Morris Water Maze tests) at 1, 2, and 3 months, respectively; 11A5 and 6E10 treatment groups showed decreased the latencies to find the platform (improved learning) compared with control group. * $P < 0.01$ vs. control group. D, E, and F: spatial probe tests (Quantification of time spent in the target zone during the probe tests) at 1, 2, and 3 months, respectively. 11A5 and 6E10 treatment groups spent much longer time in the target quadrant than the control group. * $P < 0.01$ vs. control group at 1-month. #: $P < 0.05$ vs. control group at 2, 3-month. G, H, and I: the number of times the platform was located at 1, 2, and 3 months, respectively. 11A5 and 6E10 treatment groups had much more crossings of the annulus than control groups. #: $P < 0.05$ vs. control group. 11A5 group showed little difference with 6E10 group. $n = 5/\text{group/time point}$.

Fig. 8.

ELISA results showing concentrations of Aβ in brains and peripheral blood. A: Aβ concentrations were lower in brains in the treatment groups compared with the control group. *: $P < 0.01$ vs. control group. B: The concentrations of Aβ were higher in peripheral blood in the treatment groups compared with the control group. *: $P < 0.01$ vs. control group at 1-month; #: $P < 0.05$ vs. control group at the others two months. There was no significant difference between 11A5 and 6E10 group. $n = 5$ / group/time point.