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The protective effects and underlying mechanism of an anti-oligomeric Aβ42 single-chain variable fragment antibody

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ABSTRACT

Oligomeric Aβ42 aggregates have been identified as one of the major neurotoxic components of Alzheimer's disease (AD). Immunotherapy targeted against these Aβ42 aggregates has been proposed as an appropriate therapeutic approach for the treatment of AD. Here, we report an anti-oligomeric Aβ42 single-chain variable fragment (scFv) antibody, named MO6, obtained from the human antibody library of a healthy donor. ScFv MO6 specifically recognized and bound to the oligomeric Aβ42 (Aβ42 oligomers and immature protofibrils; 18–37 kDa), and reduced their levels mainly by blocking their formation, although scFv MO6 also induced disaggregation of Aβ42 aggregates. More importantly, scFv MO6 ameliorated or attenuated Aβ42-induced cytotoxicity and increased cell viability by up to 33%. Furthermore, scFv MO6 efficiently passed through an *in vitro* blood–brain barrier (BBB) model with a delivery efficiency of 66% after 60 min post-administration. ScFv MO6 is a monovalent antibody with an affinity constant (K_D) of 5.2×10^{-6} M for Aβ42 oligomers. Molecular docking simulations of Aβ42 to scFv MO6 revealed that the approach and specific binding of scFv MO6 to oligomeric Aβ42 aggregates was achieved by conformational recognition and directed induction, which resulted in a more dynamic adaptation of Aβ42 to scFv MO6, occurring mainly in the N-terminal (3–4), middle (12–19) and C-terminal (34–42) regions of Aβ42. This binding mode of scFv MO6 to Aβ42 explains its protective effects against oligomeric Aβ42. Our findings may be applied for the design of a smaller antibody specific for Aβ42 oligermers.

1. Introduction

The occurrence and accumulation of cytotoxic Aβ42 aggregates in the human brain results in neuronal death, eventually leading to the onset of Alzheimer's disease (AD) (De-Paula, 2012; Dong et al., 2012). Oligomeric Aβ42 aggregates such as Aβ42 oligomers and immature protofibrils have been identified as the dominant molecular species responsible for the pathological processes underlying AD progression, because they exert deleterious effects on human brain cells (De Kimpe and Scheper, 2010; Robert et al., 2009). Although no effective treatment or cure for AD has been developed, numerous studies have demonstrated that antibodies against these toxic Aβ42 aggregates can prevent or even reverse their formation and protect human brain cells from cytotoxicity (Fukuchi et al., 2006; Kou et al., 2011). These antibodies exhibit specific protective effects both *in vitro* and *in vivo* (Giménez-Llort et al., 2013; Wisniewski and Goñi, 2014).

Most previous studies have investigated small single-chain variable fragment (scFv) antibodies, because they lack the Fc region responsible for activating the complement response in the body; therefore, these antibodies are considered safer than whole antibody (Huang et al., 2013). Moreover, scFv molecules are smaller, potentially facilitative, and easier to deliver into the brain than whole antibodies. Recent studies have demonstrated that anti-oligomeric Aβ42 scFv antibodies may be used to treat AD; these recombinant antibody fragments represent attractive candidates for the design of safer formulations for passive immunotherapy of AD.

Abbreviations: AD, Alzheimer's disease; CDR, complementarity determining region; scFv, single-chain variable fragment; VH, variable heavy; VL, variable light.

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Although anti-oligomeric Aβ42 scFv antibodies are now considered promising agents for the treatment of dementia, the precise mechanisms underlying their protective effects are not fully understood. Further, many studies have indicated that different anti-Aβ42 scFv molecules display different protective efficacies against oligomeric Aβ42-induced cytotoxicity. Therefore, detailed understanding of the binding and protective mechanism of anti-Aβ42 scFv against oligomeric Aβ42 is essential to enable the molecular design of more powerful anti-oligomeric Aβ42 scFv antibodies.

In a previous study, we reported an anti-oligomeric Aβ42 scFv antibody, named AS, which not only specifically binds oligomeric Aβ42 aggregates, but also attenuates their cytotoxicity (Zhang et al., 2014). In this study, we characterized another anti-oligomeric Aβ42 scFv antibody, named MO6, from the human scFv antibody library of a healthy donor (not AD patients). Here, we report the potential functional mechanism of this antibody *in vitro*. Compared to AS, scFv MO6 has a different amino acid sequence but similar protective effects on target cells *in vitro*. In this report, together with the results of our previous study on AS, we provide additional details regarding the protective effects of anti-oligomeric Aβ42 scFv antibody against oligomeric Aβ42.

2. Materials and methods

2.1. Cell cultures

Human neuroblastoma cell line SH-SY5Y cells (ATCC® CRL-2266™), human umbilical vein/vascular endothelium cell line (ATCC® CRL-1730™) HUVEC cells, and C6 glioma cells (ATCC® CCL-107™) were cultured as described previously (Zhang et al., 2014).

2.2. Aβ42 aggregates and anti-Aβ42 antibody

Various Aβ42 aggregates (oligomers, protofibrils and fibrils) were prepared from Aβ42 monomer, and confirmed by electron microscopy as described previously (Cui et al., 2010; Huang et al., 2010; Wang et al., 2010). Mouse anti-human-amyloid-β monoclonal antibody (B4) (sc-28365) was purchased from Santa Cruz Biotech, Inc.

2.3. ScFv MO6

The Human scFv antibody library of a healthy donor was constructed based on the peripheral blood leukocytes (PBL) of a healthy donor; Aβ42 oligomers-specific scFv clones were screened from the human scFv antibody library as described previously (Zhang et al., 2014). Clone MO6 that showed strong reactivity toward Aβ42 oligomers was selected and the scFv MO6 was prepared as described previously (Zhang et al., 2014). The purity of the scFv MO6 was examined by 12% SDS-PAGE.

2.4. Examination of binding specificity of scFv MO6 to four Aβ42 species by dot blot and western blot analyses

For dot-blot procedure, aliquots of each Aβ42 species (monomers, oligomers, protofibrils and fibrils) with a final concentration of 10 μM in 10 mM PBS (pH 7.4) were directly spotted onto nitrocellulose membrane by using circular template. The membrane was washed in PBST buffer (PBS containing 0.1% Tween-20), and non-specific bindings were blocked with 5% nonfat dry milk for 1 h at RT. Dots were probed with scFv MO6 for 1 h at RT, and were visualized by ECL chemiluminescence kit (Amersham Pharmacia Biotech, UK) using primary anti-His antibody (1:1000 dilution) and the secondary HRP-conjugated goat-anti-rabbit IgG (1:5000 dilution). The binding specificity of scFv MO6 to the four Aβ42 species was evaluated by visually comparing the color intensities of all the Aβ42 dots, and was semi-quantitatively determined by gray scanning analysis of dot images.

For western blot procedure, 5-μL mixture of the four Aβ42 species was resolved in 12% native-PAGE, and was transferred to polyvinylidene difluoride (PVDF) membrane. The PVDF membrane was then probed as described above. The binding specificity of scFv MO6 to all the Aβ42 species was assessed by comparing the color intensities of all the Aβ42 bands.

2.5. Determination of Aβ42 oligomer-binding capacity of scFv MO6 by sandwich ELISA

96-microwell plates were coated with 100-μL scFv MO6 at various final concentrations (1.0, 2.0×10^{-2}, 5.0×10^{-3}, 1.25×10^{-3}, 6.25×10^{-4}, 3.12×10^{-4} μM) at 4 °C overnight. The wells were blocked with 1% BSA in PBS for 1 h at RT, and then incubated with 100 μL of Aβ42 oligomers at 5.0×10^{-3} μM in PBS for 1 h at 37 °C. Negative control wells contained medium plus unrelated protein (BSA). All wells were probed with B4 antibody (1:2000 dilution, Santa Cruz, USA) followed by anti-mouse HRP IgG (1:4000) for 1 h at 37 °C, and then were developed with TMB. After 15 min of development, the absorbances of all the wells at 450 nm were monitored on an ELISA microplate reader (Thermo Fisher Scientific Inc., USA). Experiments were performed in triplicate. A scFv MO6 sample is considered positive if its OD value is not only greater than the sum of 0.2 plus the OD value of the control, but also is 2.1-fold greater than the OD value of the control.

2.6. Measurement of equilibrium dissociation constant (K_D) of scFv MO6 to Aβ42 oligomers by indirect competitive ELISA

By using serial dilutions scFv MO6 by sandwich ELISA.

K_D (the equilibrium dissociation constant between antibody and antigen) was measured according to the method established by Bertrand Friguet (Friguet, 1985, 1984). Aβ42 oligomers at 10^{-10} to 10^{-4} M in 10-fold serial dilutions were respectively mixed with scFv MO6 (1.0×10^{-7} M in 10 mM PBS, pH 7.4). After 1 h of incubation at 37 °C, 100-μL mixture per well was transferred into another well which was pre-coated with 10 μg/ml Aβ42 oligomers for 12 h at 4 °C, and incubated for additional 1 h at 37 °C. After washing with PBST buffer (PBS containing 0.1% Tween-20), the amount of the bound scFv MO6 was assessed by anti-His antibody (1:2000) followed by HRP-conjugated goat anti-rabbit IgG (1:4000) using TMB. 15 min later, the absorbance of each well was measured at 450 nm. Experiments were performed in triplicate. K_D value equals the initial concentration of free Aβ42 oligomers (M) at which concentration half of scFv MO6 bound to the coated Aβ42 oligomers.

2.7. Measurement of Aβ42 aggregates by Thioflavin T fluorescence assay

Degree and/or extent of Aβ42 aggregation was measured by Thioflavin T (ThT)-fluorescence assay (ThT-F) as described previously (Zhang et al., 2014).

2.8. Assessment of the cytoprotective efficacy of scFv MO6 against Aβ42 oligomers and protofibrils by MTT cell viability and lactate dehydrogenase (LDH) release assays

Human neuroblastoma SH-SY5Y cells were seeded in 96-well plates (Corning, USA) at approximately 1000 cells in 100 μL of medium (DMEM-F12) per well, and incubated for 24 h in 5% CO_2