A novel Aβ42 oligomer-specific antibody ameliorates the neurotoxicity of postmortem brain extracts from patients with Alzheimer's disease

Anders Sandberg[1], Rosa Crespo Rodriguez[2], Ernesto Berenjeno Correa[3], Tjado Morrema[4], Jeroen J.M. Hoozemans[4], and Wiep Scheper[3]

Introduction

Aggregation of the 42-residue peptide amyloid-β (Aβ42) in the brain is implicated as the primary driver of Alzheimer's disease (AD). However, a recent report demonstrated that only a fraction of the soluble aggregated Aβ, or oligomers, carries most of the neurotoxic potency; whereas the insoluble deposits, or plaques, are not toxic despite being a prominent feature of the disease (Hong *et al*., Acta Neuropathol., 2018).

Here we present data demonstrating the ability of a highly selective Aβ42-oligomer-targeting monoclonal antibody, ALZ-201 (Figure 1), to neutralise the neurotoxicity of postmortem brain extracts from patients with AD. The antibody was developed by immunising mice with the vaccine, ALZ-101. The vaccine and the antibody both target the same oligomeric species of Aβ. The vaccine ALZ-101 is set to enter clinical Phase 1b in Q3 2021.

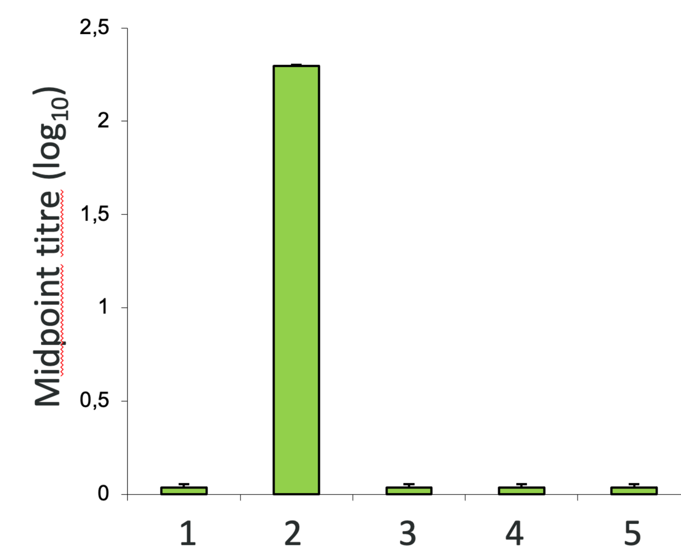

Figure 1. Titres from direct ELISAs of ALZ-201 against (1) non-aggregated Aβ42CC peptides (monomeric); (2) oligomeric Aβ42CC; (3) Aβ42 fibrils; (4) non-aggregated Aβ40CC; and (5) oligomeric Aβ40CC. "AβCC" is a protein-engineered Aβ peptide designed to only form oligomers (Sandberg et al., PNAS, 2010).

Methods

Postmortem brain tissue samples from the frontal cortex were obtained from the Netherlands Brain Bank. Frozen sections were stained with anti-Aβ (anti-Aβ a.a. 1-5) and anti-pTau (p-Ser202/p-Thr205 antibody AT8) antibodies to confirm pathology/non-pathology.

Case #	Diagnosis	Sex	Age	IHC Aβ	IHC pTau
AD01	AD	F	85	Positive	Positive
AD02	AD	M	95	Positive	Positive
AD03	AD	M	72	Positive	Positive
AD04	AD	F	80	Positive	Positive
AD05	AD	F	96	Positive	Positive
AD06	AD	F	86	Positive	Positive
AD07	AD	M	86	Positive	Positive
C01	Control	M	97	Negative	Negative
C02	Control	M	60	Negative	Negative
C03	Control	F	62	Negative	Negative

Table 1. Seven brain samples from deceased AD patients and three brain samples from deceased healthy controls were used in this study. Pathology was confirmed with anti-Aβ and anti-pTau immunohistochemical (IHC) staining.

Brain extracts (H, S and H2) were prepared following the protocol of Hong et al. (cf. Figure 2), aliquoted, snap-frozen and stored at -80 °C. Total protein content of the brain extracts was determined using the Pierce BCA Protein kit (Thermo Scientific). Aβ was quantitated with the mesoscale discovery (MSD) platform using the Aβ peptide panel 1 kit (Aβ42, Aβ40, Aβ38) according to the manufacturer's instructions.

Immunoprecipitation (IP) was carried out by incubating brain extracts with antibody (ALZ-201, 4G8, or an isotype control) overnight at 4 °C, then adding Protein G Sepharose beads followed by centrifugation. Supernates were snap frozen in N_2 (l) and stored at -80 °C.

Methods, cont.

On day 18.5 post fertilisation, mouse embryos were obtained by cesarean section and used for the preparation of mouse primary neuronal cultures. On the 17th day in vitro, cells were treated with brain fractions for 24h, fixed, and labelled with antibodies towards vGlu1 (SySy), NF-H (Eurogentec), and MAP2 (Abcam). Nuclei were stained with DAPI (Brunschwig Chemie). Images were analysed by automated microscopy.

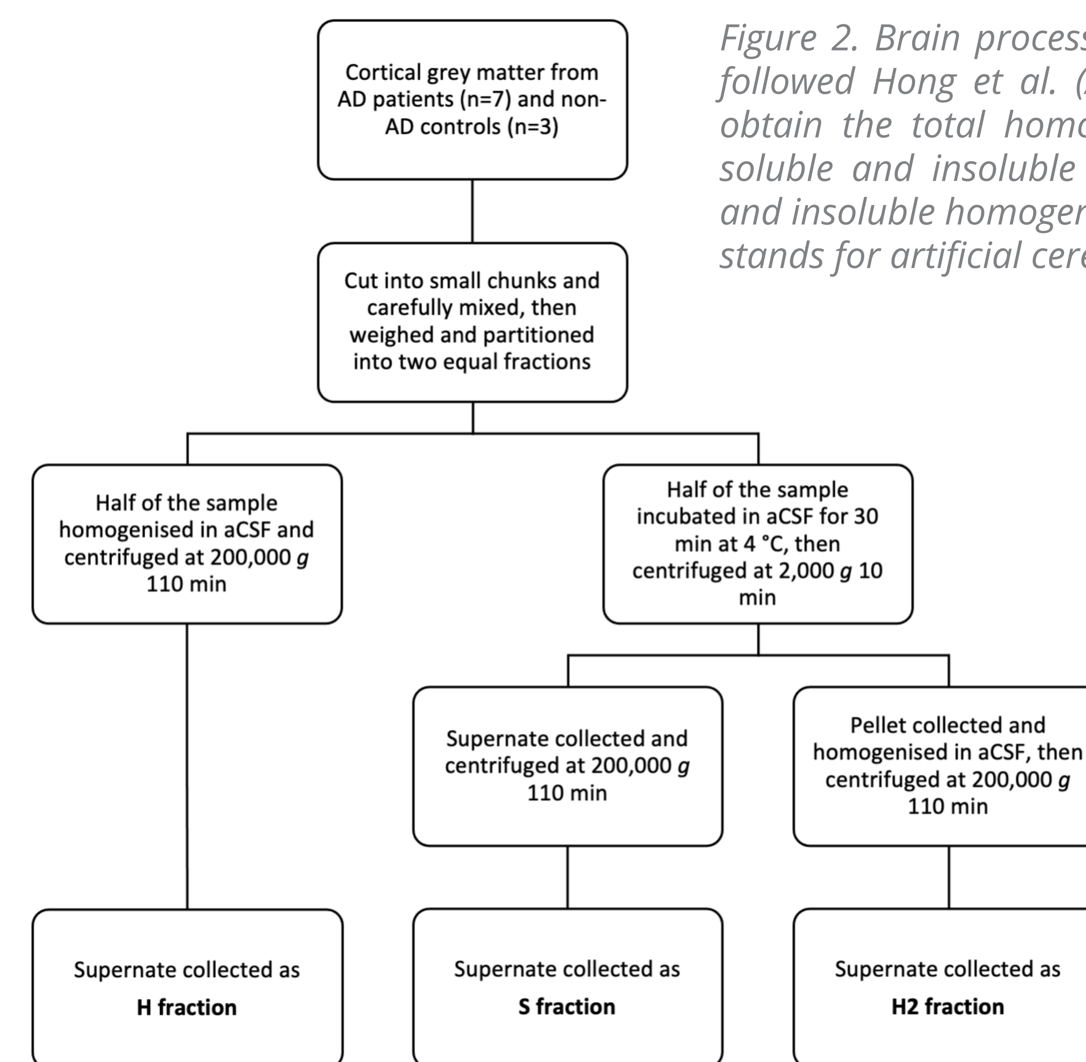

Figure 2. Brain processing flow-chart. The protocol followed Hong et al. (Acta Neuropathol., 2018) to obtain the total homogenised fraction containing soluble and insoluble Aβ (H), soluble Aβ only (S), and insoluble homogenised Aβ only (H2). Here, aCSF stands for artificial cerebral spinal fluid.

Results, biochemical characterization

The total protein content was within the same range for all brain extract samples (not shown). The amount of Aβ was higher for AD cases compared to controls (Figure 3, Left), as expected, but exhibited high variability. IP with the positive control antibody, 4G8, strongly depleted all Aβ, whereas the ALZ-201 antibody and the negative isotype control antibody did not significantly affect total Aβ levels (Figure 3, Right), indicating that the ALZ-201 antibody targets a low-abundant oligomer.

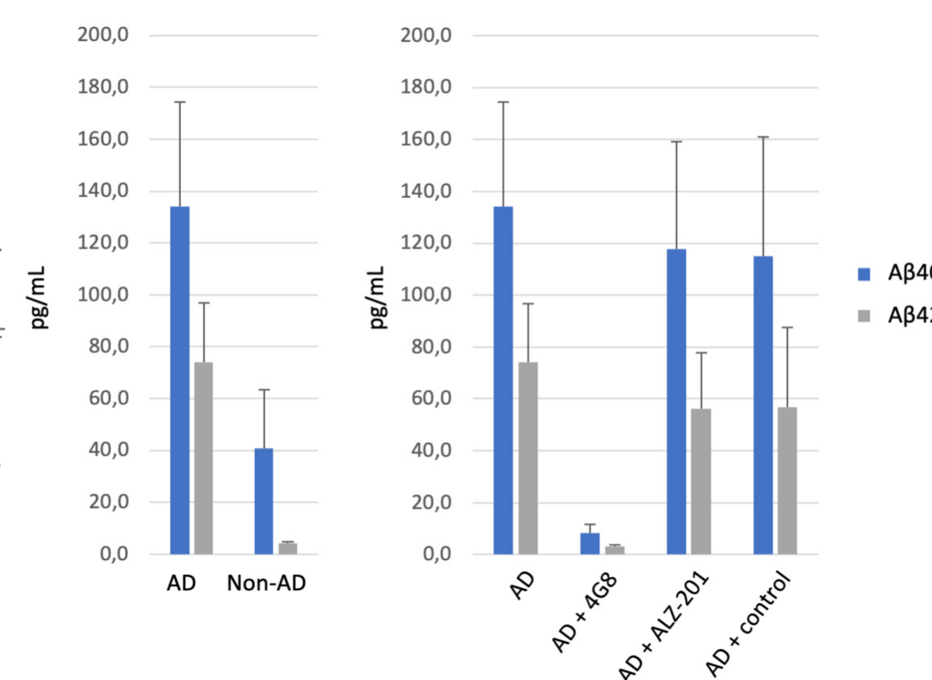

Figure 3. Left panel: MSD quantification of Aβ40 and Aβ42 in the H fraction obtained from AD patients (n=4; first four brains in Table 1) and non-AD controls (n=3). Right panel: MSD quantification of Aβ40 and Aβ42 in brain fraction H from four brain samples (first four brains in Table 1) immunoprecipitated with 4G8 (removes all Aβ), ALZ-201 (specific for a subtype of oligomeric Aβ42) and isotype control (does not bind any Aβ).

Results, neurotoxicity assessment

Treatment of primary neurons with brain extracts showed that the H and S fractions from AD patients were more toxic than the H2 fraction and non-AD controls. Results were more robust for the H fraction, possibly due to the fewer processing steps for this fraction. Here we present the main results for the H fraction only.

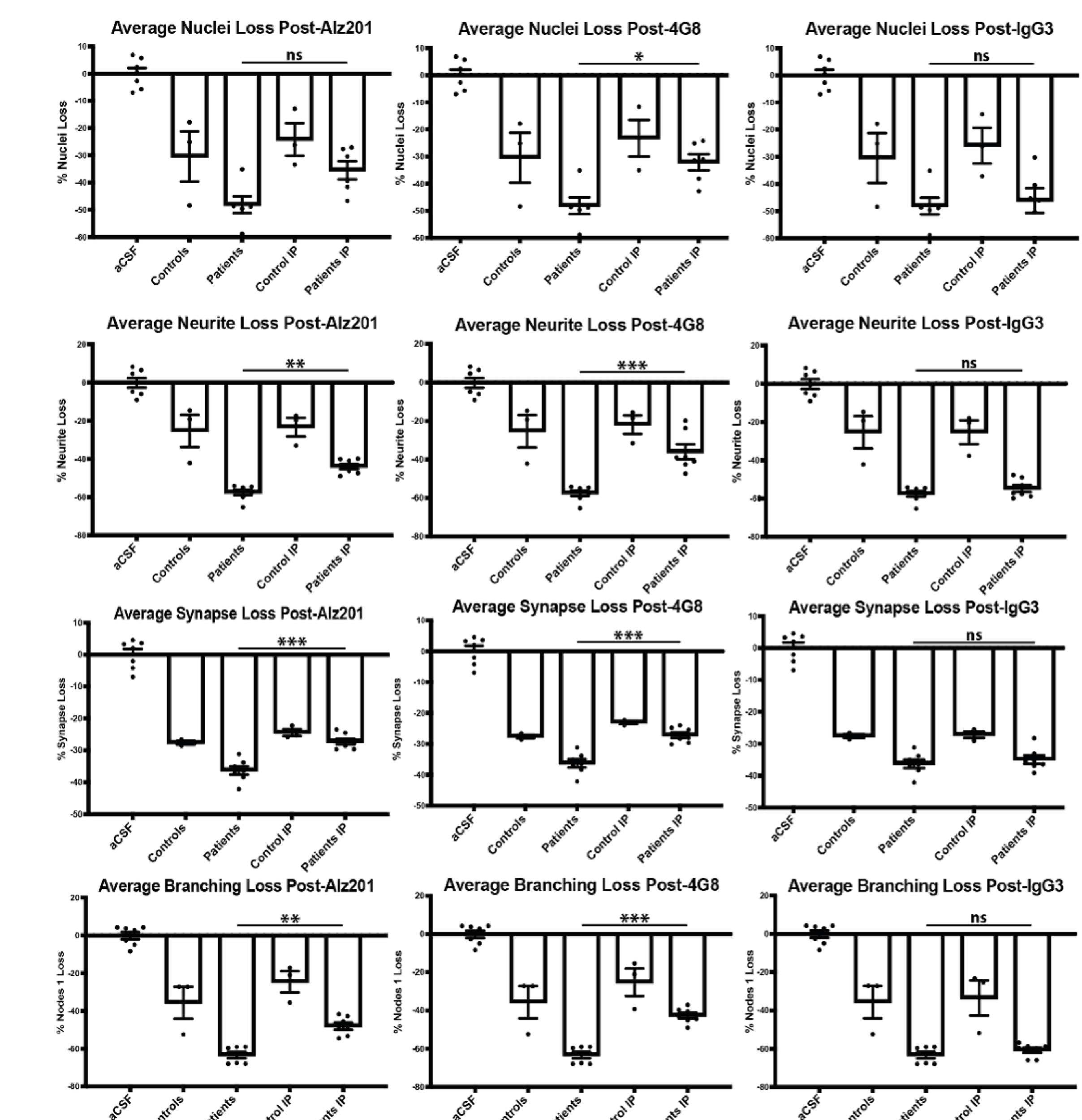

*Figure 4. Average nuclei, neurite, synapse and branching loss assessed by an automated imaging platform after treatment of primary mouse neurons with postmortem whole brain extracts (fraction H obtained from the samples in Table 1). ALZ-201 and 4G8 are able to ameliorate the toxicity from the AD extracts, whereas the non-Aβ reactive IgG control did not have an effect. Only the nuclei loss did not reach statistical significance. N.s. stands for "not significant" and indicates p>0.05, * indicates p≤0.05, ** p≤0.01, and *** p≤0.001.*

Conclusions

ALZ-201 has a binding profile that differs from known antibodies. Although it only targets a very small fraction of all Aβ, it has a profound effect on the neurotoxicity of patient-derived Aβ. This suggests it has extremely high selectivity for the toxic form of the Aβ peptide. Since this natural toxic form of Aβ is very low in abundance, this feature may be a critical attribute for achieving a true therapeutic effect in actual patients.

Affiliations

(1) Alzinova AB, Gothenburg, Sweden **(2)** Dept. of Clinical Chemistry, Amsterdam UMC location VUmc, Netherlands **(3)** Dept. of Human Genetics, Amsterdam UMC location VUmc, Netherlands, and **(4)** Dept. of Neuropathology, Amsterdam UMC location VUmc, Netherlands.

Presented at the Alzheimer's Association International Conference®
Denver, USA and Online
July 26-30, 2021