

Reduction of amyloid load and cerebral damage in a transgenic mouse model of Alzheimer's disease by treatment with a β -sheet breaker peptide¹

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SPECIFIC AIMS

Deposition of cerebral amyloid plaques is a hallmark feature of Alzheimer's disease (AD) and reduction of amyloid load is widely regarded as the most promising therapy for the disease. We investigated the ability of a five-residue β -sheet breaker peptide (iA β 5p) with improved pharmacological properties to reduce amyloid deposition and cerebral damage in vivo using two different transgenic mice models of AD.

PRINCIPAL FINDINGS

1. A β -sheet breaker peptide reduces amyloid load in vivo

Experiments were carried out in a double transgenic animal model of AD, overexpressing human APP with the London mutation (V717I) and human PS1 bearing the A246E mutation. These animals develop many of the pathological features of AD, including extensive deposition of amyloid plaques starting at 6 months of age, neuritic dystrophy, astrogliosis, and some degree of tau pathology. A group of 6- to 7-month-old animals were treated by intra-cerebroventricular (i.c.v.) infusion of 2.5 mg of iA β 5p for 8 wk. Immunohistological analysis of hippocampus revealed a 67.3% reduction of amyloid burden in animals treated i.c.v. with iA β 5p compared to controls. A second group of 8- to 9-month-old animals was treated by intraperitoneal (i.p.) injection of 1 mg of peptide three times a week for 8 wk. Animals treated by i.p. with iA β 5p showed a 46.5% reduction of amyloid load compared with control animals treated with vehicle (**Fig. 1**). The overall effect of iA β 5p was statistically significant as studied using a two-way analysis of variance (ANOVA). The immunological reaction to the treatment was studied in the i.p. treated animals. No antibodies against either iA β 5p, A β 40, or A β 42 were detected in sera at the beginning or end of treatment. This finding suggests that the effect of iA β 5p was likely due to direct inhibition and/or dissolution of amyloid plaque formation and

not to an indirect antigenic response, as found during A β immunization experiments. These results were confirmed by an independent experiment in a single transgenic model expressing human APP_{V717I}. A group of eleven 15-month-old animals treated four times a week with i.p. injections of 1 mg iA β 5p over 3 months showed a significant reduction of amyloid plaques vs. animals treated with vehicle. Histological analysis indicated a 45.3% and 29.4% reduction of amyloid load in cerebral cortex and hippocampus, respectively. Levels of sA β were measured in brain by sandwich ELISA after homogenization. sA β 1–40 and sA β 1–42 were decreased by 43.4% and 25.8%, respectively. This effect was significant ($P=0.036$) as analyzed by two-way ANOVA, considering the A β species and treatment as the variables.

2. A β -sheet breaker peptide reduces neuronal death induced by amyloid in vivo

We investigated whether the reduction of amyloid translated in a lower level of neuronal damage in the brain of these mice. Brain sections from the experiment with double transgenic animals were stained with an antibody against a specific postmitotic neuronal marker named NeuN (**Fig. 2**). Amyloid caused a severe disruption of the normal cytoarchitecture of neurons in subiculum and hippocampus; cells surrounding plaques showed some typical features of dying neurons, including pyknotic bodies or alteration in the cell body shape (**Fig. 2a**). Neurons were counted on i.p. and i.c.v. treated animals. Animals treated by i.c.v. and i.p. administration of iA β 5p shown a 20.7% (**Fig. 2b**) and 16.9% (**Fig. 2c**) increase in neuronal survival in the subiculum area, respectively. This effect was statistically significant as studied by an unpaired *t* test. Similar results were observed when the slides were stained by

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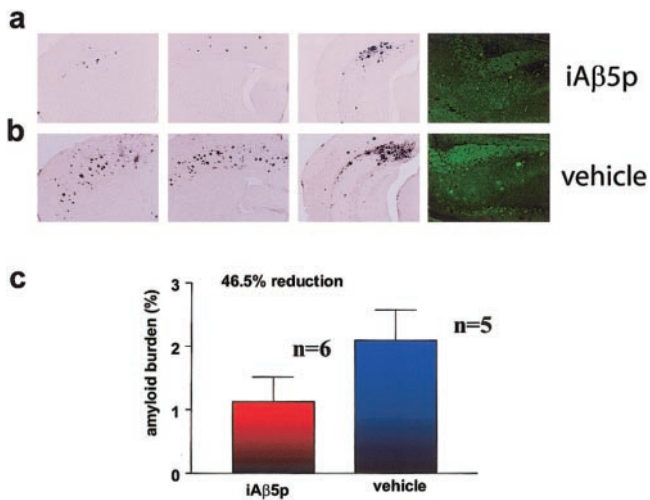


Figure 1. In vivo reduction of amyloid load by i.p. administration of iAβ5p on AD transgenic mice. Animals treated with iAβ5p (*a*) showed less amyloid than controls (*b*) by immunohistology using a polyclonal antibody and by thioflavine S staining (right panels, *a*, *b*). *c*) Quantitative analysis of amyloid load in hippocampus showed a 46% reduction of amyloid load in iAβ5p-treated animals vs. controls. Each bar represents the average \pm SE among the animals used per group (*n*). The effect was significant as analyzed by two-way ANOVA ($P < 0.05$).

cresyl violet and cells stratified by size and morphology to analyze the number of neurons in the animals without and with treatment. A 24.5% and 23.7% higher number of neurons were observed in the double transgenic animals treated with iAβ5p by i.c.v. and i.p. routes, respectively. We also investigated whether amyloid reduction resulted in a decrease of brain inflammation. Amyloid deposits were found surrounded by astrocytes and activated microglia, showing a distribution similar to that observed in human AD brain. A clear colocalization of reactive astrocytes and activated microglia with amyloid plaques was observed in subiculum, hippocampus, and neocortex. The extent of astrogliosis and microglial activation was directly dependent on the amyloid load, and treatment with iAβ5p resulted in a marked reduction of brain inflammation.

3. Rapid brain uptake of the β-sheet breaker peptide

A major drawback with the use of peptides as drugs in central nervous system diseases is their rapid metabolism by proteolytic enzymes and their poor blood–brain barrier (BBB) permeability. To minimize protease degradation, iAβ5p is protected by acetylation in the amino terminus and amidation in the carboxyl terminus. Indeed, the peptide is very stable in human plasma in vitro, showing little or no degradation within 24 h of incubation at 37°C. However, pharmacokinetic studies in rats showed in vivo degradation of the peptide and calculated a half-life of 37 ± 5 min. Biodistribution of the labeled peptide shows that the brain contained a higher amount of radioactive material than most of the

organs, excluding those related to metabolism and excretion. Detailed brain uptake experiments were performed with mice using brain perfusion and capillary depletion techniques. iAβ5p penetrated into the brain at a rate $> 300 \mu\text{l/g}$, suggesting a high capability of the peptide to cross the BBB. Under these conditions, no peptide degradation was observed, suggesting that the cleavage of the peptide detected after intravenous injection was carried out mainly in some of the systemic organs. The majority of iAβ5p taken up by brain ($> 90\%$) was found in the parenchyma fraction, demonstrating complete passage across the BBB. We estimate that $> 0.09\%$ of the injected iAβ5p was recovered intact in the brain.

CONCLUSIONS

Our results show that a β-sheet breaker peptide modified to increase stability against proteolytic degradation is able to induce a dramatic reduction in amyloid deposition and cerebral damage in transgenic animal models of AD. An issue debated for a long time in the AD field is the role of Aβ and amyloid plaques in the disease pathogenesis. Most of the evidence supports the amyloid hypothesis, which proposes that Aβ production, misfolding, and aggregation are central in the disease and are the direct cause of cerebral damage and clinical symptoms (**Fig. 3**). An alternative view is that amyloid plaques are an epiphenomenon and simply

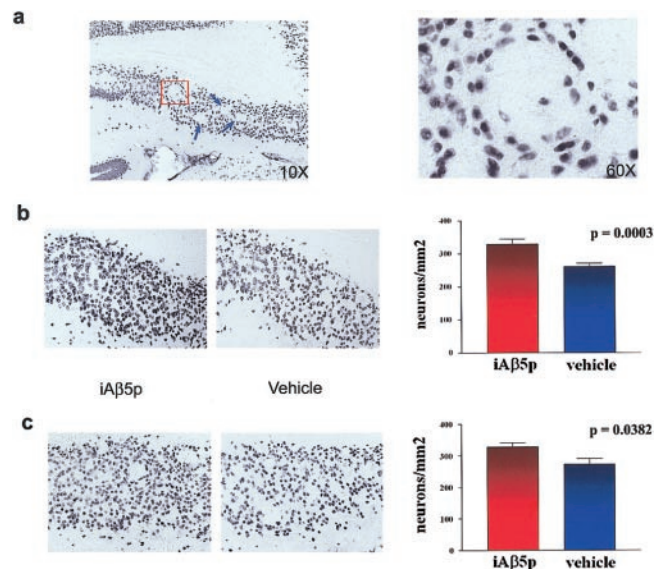
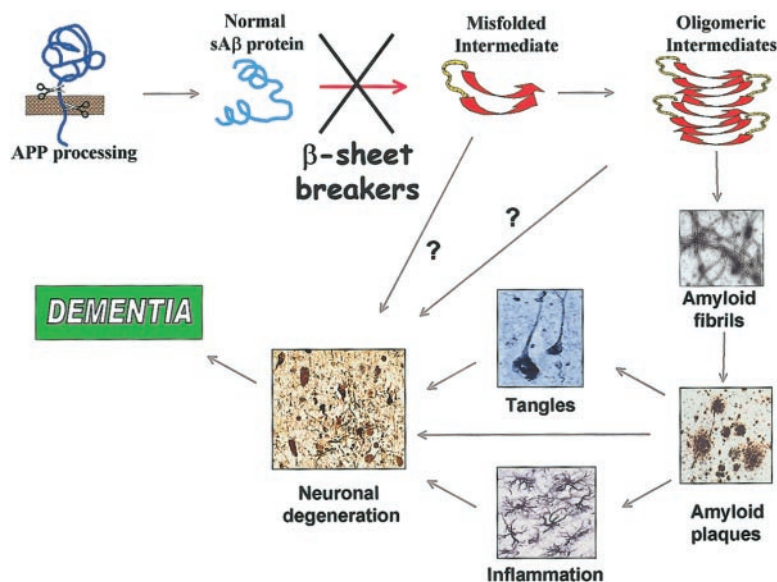


Figure 2. Increase in neuronal survival by iAβ5p treatment. *a*) Staining of 40 μm cryostat sections with an antibody against a specific neuronal marker showed a clear neuronal disturbance in subiculum at low (upper panel, left) and high magnification (upper panel, right). *b*, *c*) Neuronal counting in subiculum on i.c.v. and i.p. treated animals, respectively. A reduction in the number of neurons was observed in controls compared to iAβ5p-treated animals (*b*, *c*). Statistical analysis (*t* test) showed a significant effect in i.c.v. ($P = 0.003$) and i.p. ($P = 0.039$) treatments.

Figure 3. Schematic representation of the amyloid hypothesis for the progression of AD and the potential site of action of β -sheet breaker peptides. In the amyloid hypothesis, the misfolding and aggregation of the otherwise normal soluble A β protein is the key pathological event in the pathogenesis of AD. Either amyloid plaques or their early intermediates may directly induce neurodegeneration or through the induction of brain inflammation or neurofibrillary tangles. β -Sheet breaker peptides are designed to prevent and reverse the early misfolding of A β .



represent a manifestation of the cerebral damage induced by an unknown mechanism. It is widely accepted that the development and *in vivo* evaluation of amyloid inhibitors will help clarify the role of amyloid in AD pathogenesis. In this study, we demonstrate for the first time that a treatment directed to reduce amyloid load results in an increase in neuronal survival, suggesting that amyloid plaques are indeed associated with neuronal loss in AD. Our results do not necessarily suggest that mature amyloid plaques are the toxic agent, since it is possible that an oligomeric or protofibrillar intermediate rather than the deposited fibrils are the culprit in neuronal loss (Fig. 3). In addition, our data do not necessarily suggest that amyloid plaques (or their precursors) directly induce neuronal death. Alternatively, an indirect process activated by amyloid might be the immediate cause of neuronal apoptosis. It has been proposed that a chronic brain inflammation triggered by the accumulation of amyloid plaques might be the intermediate process. Our data suggest that a treatment designed to reduce amyloid burden also decreases astrogliosis and thereby reduces cerebral damage in AD. Decreased neuronal loss and brain inflammation should translate into clinical benefit for the patients.

Although several peptides have been used to treat

diverse diseases, the drug potentiality of peptides is limited by their rapid degradation in biological fluids and tissues, their immunogenicity, and their poor bioavailability. In the case of brain disorders, the compound must be able to penetrate the tight and selective BBB. We have found that small chemical modifications can dramatically increase peptide stability in blood. We also found that administration of relatively large doses of iA β 5p did not elicit antibody production during the 2 months of treatment. This is not completely surprising, because short peptides are poorly immunogenic in the absence of adjuvants. The surprising result is the finding that iA β 5p crosses the BBB at a rate higher than most proteins and peptides known to be selectively taken up by the brain. The mechanism responsible for the high rate of brain penetration of this peptide is under evaluation.

In conclusion, the present study reports a short β -sheet breaker peptide with acceptable pharmacological properties and the ability to reduce amyloid load and cerebral damage by systemic administration in a transgenic model of AD. These results open the way for clinical trials to evaluate the ability of such peptides to reduce cerebral amyloid burden and disease progression in AD patients.

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