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Disease modifying effect of chronic oral treatment with a neurotrophic peptidergic compound in a triple transgenic mouse model of Alzheimer's disease

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ABSTRACT

Besides the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles, neurogenesis and synaptic plasticity are markedly impaired in Alzheimer's disease (AD) possibly contributing to cognitive impairment. In this context, neurotrophic factors serve as a promising therapeutic approach via utilization of regenerative capacity of brain to shift the balance from neurodegeneration to neural regeneration. However, besides more conventional "*bystander*" effect, to what extent can neurotrophic compounds affect underlying AD pathology remains questionable. Here we investigated the effect of chronic oral treatment with a ciliary neurotrophic factor (CNTF) derived peptidergic compound, P021 (Ac-DGGL^{A}G-NH_2), on disease pathology both at moderate and severe stages in a transgenic mouse model of AD. 3xTg-AD and wild type female mice were treated for 12 months with P021 or vehicle diet starting at 9–10 months of age. A significant reduction in abnormal hyperphosphorylation and accumulation of tau at known major AD neurofibrillary pathology associated sites was observed. The effect of P021 on Aβ pathology was limited to a significant decrease in soluble Aβ levels and a trend towards reduction in Aβ plaque load in CA1 region of hippocampus, consistent with reduction in Aβ generation and not clearance. This disease modifying effect was probably via increased brain derived neurotrophic factor (BDNF) expression mediated decrease in glycogen synthase kinase-3-β (GSK3β) activity we found in P021 treated 3xTg-AD mice. P021 treatment also rescued deficits in cognition, neurogenesis, and synaptic plasticity in 3xTg-AD mice. These findings demonstrate the potential of the neurotrophic peptide mimetic as a disease modifying therapy for AD.

Introduction

Alzheimer's disease (AD) is the most common age-dependent neurodegenerative disorder which contributes significantly to health care burden in industrialized countries, especially because of lack of an effective therapy due to its multifactorial and heterogeneous nature and involvement of several different etiopathogenic mechanisms (Iqbal et al., 2005; Iqbal and Grundke-Iqbal, 2010, 2011). AD is the sixth most prevalent cause of mortality in the U.S. and the leading cause of dementia, affecting over 5 million Americans and 35 million people worldwide (Hebert et al., 2013; Iqbal and Grundke-Iqbal, 2011). The number of Americans with AD is projected to be 13.5 million by 2050 unless a drug is developed that can prevent or inhibit this disease (Hebert et al., 2013). Histopathologically, AD is characterized by two major lesions: amyloid as diffuse and neuritic plaques composed of amyloid beta (Aβ) peptide, and neurofibrillary tangles composed of hyperphosphorylated tau protein (Glenner and Wong, 1984; Grundke-Iqbal et al., 1986). Currently, the four FDA approved drugs (donepezil, galantamine, rivastigmine, and memantine) available for AD treatment only provide symptomatic benefit with little effect on the underlying pathology (Frisardi et al., 2010; Panza et al., 2009a,b, 2012). Obviously, there is impending urgency to find an effective disease-modifying therapy.

Independent of the various etiopathogenic mechanisms involved in AD, they all cause neurodegeneration. Thus, a successful therapeutic strategy for AD may include both inhibition of neurodegeneration as well as stimulation of regeneration in affected areas of the brain. This shift of balance from neurodegeneration to neural regeneration can be achieved with molecules that promote both neurogenesis and neuronal

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and synaptic plasticity (Iqbal and Grundke-Iqbal, 2011). By virtue of their neuroprotective and neurogenic capabilities demonstrated in animal models of neurodegeneration (for review, see Dawbarn and Allen, 2003), neurotrophic factors represent a promising therapeutic approach for AD. Many studies have shown that neurotrophic factor based approach for AD can ameliorate deficits in neurogenesis, synaptic plasticity, and cognition (Blanchard et al., 2010b; Blurton-Jones et al., 2009; Bolognin et al., 2012; Garcia et al., 2010; Kiyota et al., 2011; Nagahara et al., 2009). However, it remained questionable if this strategy could have an effect on underlying Aβ and tau pathologies.

In the present study, we used a blood–brain barrier (BBB) permeable CNTF derived peptidergic compound, Peptide 021 (P021), which exerts it's neurogenic and neurotrophic effect mainly by inhibiting leukemia inhibitory factor (LIF) signaling pathway and enhancing brain derived neurotrophic factor (BDNF) expression by increasing its transcription (Bolognin et al., 2014; Li et al., 2010). We show that chronic treatment with P021, administered in diet to triple transgenic AD (3xTg-AD) mice, can not only restore impairments in neurogenesis, dendritic and neuronal plasticity, and cognition at moderate stage of the disease but also strongly attenuate tau pathology both at moderate and severe stages of the disease. P021 also exerted a marginal reduction in Aβ pathology at moderate stage of the disease. This disease modifying effect of P021 was probably due to the increased BDNF expression-mediated reduction in glycogen synthase kinase-3β (GSK3β) activity we found in the P021-treated 3xTg-AD mice, and further confirmed in P021-treated primary cultured cortical neurons.

Materials and methods

Design and synthesis of P021

Fig. 1A shows the structure of the peptidergic compound P021 (Ac-DGGL^{A}G-NH_2; mol. wt. of 578.3) which corresponds to a biologically active region of human CNTF (amino acid residues 148–151) to which adamantylated glycine was added to increase its stability and lipophilicity (Blanchard et al., 2010a; Chohan et al., 2011; Li et al., 2010). The peptide was synthesized and purified by reverse phase HPLC to >96% purity, as described previously (Li et al., 2010). The sequence of the peptide was confirmed by mass spectrometry.

Stability of P021 in plasma and gastric and intestinal juices and blood–brain barrier permeability

The studies on the plasma stability and stability in gastric and intestinal juices of P021 were performed by EVER NeuroPharma GmbH, Unterach, Austria. The plasma stability was analyzed in human pooled plasma in PBS (1:1) using different concentrations of P021 (1 μM, 1 mM, and 40 mM). The acetonitrile with internal standard albendazole was used as stop solution, and peptide concentrations were measured by HPLC. The plasma concentration of P021 reached 50% of the initial amount in 180–200 min (corresponding to a half life of >3 h). For 1 L artificial gastric juice, 2 g of NaCl and 3.2 g of pepsin were dissolved in 100 mL water; 80 mL of 1 M HCl was then added, pH was adjusted to 2.5 ± 0.5 and the final volume was made up to 1 L with water. The P021 was stable (>90%) in artificial gastric juice as analyzed up to 30 min. For 1 L artificial intestinal juice, 6.8 g sodium dihydrophosphate and 10 g Pankreatin were dissolved in 380 mL 0.1 N NaOH. The pH was adjusted to 7.5 ± 0.1 and the final volume was made up to 1 L with water. The P021 was found to be stable (>95%) in artificial intestinal juice up to 2 h.

P021 was expected to be BBB permeable as it is a four amino acid fragment with adamantylated glycine (enhances lipophilicity) added to the C-terminal of an 11-mer parent CNTF peptide, Peptide 6 (P6), which we previously showed to be BBB permeable (Chohan et al., 2011). The BBB studies on P021 were carried out through a commercial service (APREDICA, Watertown, MA). Adult mice (9–11 month old C57BL/6) were given a single i.p. injection of 1.5 mg/0.1 mL P021/mouse. Animals were bled 10 and 30 min post injection and plasma was isolated; 10 and 30 min post injection, each animal was transcardially perfused with PBS followed immediately by the removal of the brain and its homogenization in 1 mL PBS. The brain concentrations, as analyzed by LC/MS/MS, were 28 ± 8.5 ng/mL and 2.35 ± 1.7 ng/mL 10 and 30 min post i.p. injection respectively. The brain:plasma ratio increased by 67% from 10 to 30 min. In a previous study, we showed that P021 acted via dose dependent competitive inhibition of LIF activity towards LIF/CNTF receptor at as low as 0.1 nM ($p < 0.05$ at 10 nM P021) concentration as measured by phosphorylation of STAT3 at Tyr705 (Li et al, 2010). In the current study (Fig. 1B), we found that oral administration of P021 in the diet decreased the phosphorylation of STAT3 at pTyr705 in the hippocampus of both WT and 3xTg-AD mice after 6 months of treatment (Figs. 1C & D). This provided evidence that sufficient amount of P021 crossed the BBB to exert it's effect in the brain.

Animals and housing

The 3xTg-AD mouse represents one of the most biologically relevant animal models described so far as it replicates all histopathological and behavioral hallmarks of AD (Oddo et al., 2003). The 3xTg-AD mice harbor three AD-related genetic loci: human PS1 M146V, human APP_{SWE} KM670/671NL, and human tau P301L. These mice develop both amyloid plaques and neurofibrillary tangle-like pathologies in a progressive and age-dependent manner, starting at ~9 and ~12 months, respectively, but show cognitive impairment as early as ~5 months (Billings et al., 2005; Oddo et al., 2003). Several other aspects of pathology also mimic AD pathophysiological changes and clinical phenotypes such as impairment of neurogenesis and synaptic plasticity, and cognitive decline, all of which precede Aβ and tau pathologies (Billings et al., 2005; Oddo et al., 2003).

The homozygous 3xTg-AD mice were obtained from Dr. Frank LaFerla (University of California, Irvine) through the Jackson Laboratory (New Harbor, ME, USA). The background of the 3xTg-AD mice is a hybrid 129/Sv × C57BL/6. The non-transgenic wild type (WT) mice used were from the same strain and genetic background and were also obtained from the Jackson Laboratory. Mice were housed and bred in accordance with approved protocols from our Institutional Animal Care and Use Committee (IACUC), according to the PHS Policy on Human Care and Use of Laboratory animals (revised January, 2013). This study was performed on homozygous 3xTg-AD and WT female mice. Female 3xTg-AD mice were chosen because previous studies demonstrated higher Aβ burden and worse cognitive performance in female 3xTg-AD mice compared to males (Carroll et al., 2010; Clinton et al., 2007; Hirata-Fukae et al., 2008). Mice were group-housed (4 animals per cage) with a 12:12 h light/dark cycle and with ad libitum access to food and water.

Treatment of animals with P021

The female 3xTg-AD mice (9–10 months old) (n = 15–16) and age and gender matched WT controls (n = 15–16) were treated orally with P021 or vehicle diet for 12 months. Treatment was administered as 60 nmol peptide/g formulated diet (Research Diets; New Brunswick, NJ). The vehicle-treated control animals received the same diet but without the peptide. On average, each mouse consumed ~2.7 g diet/day. The animals were behaviorally tested after 6 months of treatment (15–16 months of age). First, general behavioral battery of tests was done, and then cognitive tests were carried out. After completion of the behavioral task, half of the animals (n = 7–8/group) were perfused and brain tissue was collected for immunohistochemical and biochemical analysis. The remaining animals were continued on Peptide 021/vehicle diet for another 6 months, and were sacrificed at 21–22 months of age for immunohistochemical and biochemical analysis (Fig. 1B). No