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Targeting soluble Aβ peptide with Tramiprosate for the treatment of brain amyloidosis

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Abstract

Amyloid β-peptide (Aβ) is a major constituent of senile plaques in Alzheimer's disease (AD). Neurotoxicity results from the conformational transition of Aβ from random-coil to β-sheet and its oligomerization. Among a series of ionic compounds able to interact with soluble Aβ, Tramiprosate (3-amino-1-propanesulfonic acid; 3APS; Alzhemed™) was found to maintain Aβ in a non-fibrillar form, to decrease $A\beta_{42}$-induced cell death in neuronal cell cultures, and to inhibit amyloid deposition. Tramiprosate crosses the murine blood-brain barrier (BBB) to exert its activity. Treatment of TgCRND8 mice with Tramiprosate resulted in significant reduction (∼30%) in the brain amyloid plaque load and a significant decrease in the cerebral levels of soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ (∼20–30%). A dose-dependent reduction (up to 60%) of plasma Aβ levels was also observed, suggesting that Tramiprosate influences the central pool of Aβ, changing either its efflux or its metabolism in the brain. We propose that Tramiprosate, which targets soluble Aβ, represents a new and promising therapeutic class of drugs for the treatment of AD.

Keywords: Aβ; Alzheimer's disease; Therapeutic; Glycosaminoglycans; Amyloid; hAPP transgenic mice

1. Introduction

The presence of amyloid deposits in the brain is one of the major histopathological characteristics of Alzheimer's disease (AD) [55]. The amyloid cascade hypothesis proposes that the amyloid β-peptide (Aβ), a major component of amyloid plaques, is causally related to AD neurodegeneration and is a promising therapeutic target for disease-modifying treatment [21,22].

The Aβ peptide is produced from the sequential post-translational processing of the amyloid precursor protein (APP) by β- and γ-secretases [41]. Familial AD (FAD) mutations within the human APP gene have been linked to AD [18]. These mutations appear to cause the disease by increasing the processing of APP thereby augmenting the total levels of Aβ or specifically favoring the production of $A\beta_{42}$ [8,45,46]. The more hydrophobic $A\beta_{42}$ peptide has been shown to be more fibrillogenic in vitro, to favor the formation of assembly states thought to mediate neurotoxicity, and to facilitate the process of amyloid plaque deposition [2,3,19,25,51]. Other FAD mutations have been linked to the presenilin (PSEN) 1 and 2 genes and appear to favor the production of $A\beta_{42}$ peptides [31,40,47,48]. Importantly, mice harboring APP and/or PSEN1/PSEN2 with FAD mutation(s)

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show a progressive increase in Aβ levels and develop pathological [11,14] and behavioral changes reminiscent of those observed in AD [5,10,23].

Current anti-amyloid strategies are aimed at blocking the processing of APP by targeting the β- or γ-secretase activities, the formation of amyloid fibrils, and amyloid-associated neurotoxicity [28]. Other recent approaches have attempted to upregulate either the processing of APP by the α-secretase (non-amyloidogenic pathway) or the clearance of the Aβ peptides and associated amyloid deposits from the brain [6]. Anti-inflammatory agents with an effect on APP processing are also under investigation [32,49,54]. Several other approaches have been shown to reduce the amyloid load in transgenic animal models [1,9,42] with a favorable effect on cognitive function [4,24,33,37].

Proteoglycans, a prominent constituent of amyloid deposits, are implicated in amyloid fibril formation [20]. The sulfated glycosaminoglycans (GAGs), a component of the proteoglycans, contribute to fibrillogenesis by promoting the transition of Aβ from a random-coil to a β-sheet rich conformation and protecting the fibrillar protein from proteolysis [7,20,26,27,50]. We have screened a series of low-molecular weight (LMW) molecules that mimic the ionic properties required for the binding of GAGs to Aβ. Our previous work revealed that such LMW molecules cross the blood-brain barrier (BBB), are anti-fibrillogenic, and diminish Aβ-induced toxicity in SH-SY5Y neuronal cell cultures [15].

Here we report that a specific ionic compound, Tramiprosate (3-amino-1-propanesulfonic acid; 3APS; Alzhemed™), binds preferentially to soluble Aβ, maintains Aβ in a random-coil/α-helical rich conformation, and reduces the amyloid burden in TgCRND8 transgenic mice that develop early-onset, aggressive brain amyloidosis [5,24]. Targeting soluble Aβ in vivo resulted in a dose-dependent reduction in both the soluble and fibrillar amyloid burden in these mice. This new class of drug represented by Tramiprosate holds promise for the treatment of AD.

2. Materials and methods

2.1. Binding to soluble and fibrillar Aβ peptides

For binding to soluble Aβ peptides, the pH of an aqueous solution of Aβ (20 μM) alone or with test compound (200 μM) was adjusted to 7.4 ± 0.2 with 0.1% NaOH. The mass spectra were obtained by introducing the test solution into the electrospray source by direct infusion using a syringe pump at a flow rate of 25 μl/min, and scanning from 100 to 2100 Da in the positive mode. Analysis was performed using Micromass Q-Tof Micro mass spectrometer equipped with a Waters 2795 sample manager. MassLynx 4.0 was used for data processing and analysis. The total amount of unbound (free) peptide and compound-bound (bound) peptide were calculated using peak heights of each species. The percent bound peptide was expressed as the ratio percent of bound over the total peptide. Average binding (mean ± S.D.) was calculated from four measurements.

For binding to fibrillar $A\beta_{40}$, the compound (20 μM) was incubated with pre-formed fibrillar $A\beta_{40}$ (50 μM) in 1 mM Tris buffer at 37 °C for 1 h. Following centrifugation at 21,000 × *g* for 20 min, concentration of the test compound in the supernatant was determined by LC-MS. Standard solutions for the calibration curve were prepared from a 10 mM Tramiprosate stock solution by serial dilution with 1 mM Tris buffer. The slope and *y*-intercept were used to determine the concentration of Tramiprosate present in test samples.

2.2. Circular dichroism analysis

Doses of Tramiprosate (Aβ:Tramiprosate; 1:1, 1:10, 1:20) or 2-aminoethanesulfonic acid (2AES, taurine) (Aβ:taurine; 1:1, 1:2, 1:5, 1:10, 1:20) were added to 100 μg of $A\beta_{40}$ prepared in 0.02 M Tris acetate buffer, pH 7.4, 0.14 M NaCl, 0.005% sodium azide and incubated with continuous mixing for 48 h. The test solutions were scanned by circular dichroism (CD) using a Jasco J-715 spectropolarimeter (Jasco Corp., Japan) taking readings between 190 and 240 nm, with a resolution of 0.1 nm and a bandwidth of 1 nm.

2.3. Neuroprotection in primary neuronal culture

Primary cultures of neurons were isolated from 18-day-old fetal Sprague–Dawley rats. Hippocampi and frontal cortices were dissected out and exposed to 0.025% Trypsin-EDTA for 15 min at 37 °C. Neurons were resuspended in complete Neurobasal media (0.5 ml L-glutamine, 25 μM glutamate, 25 μM 2-mercapto-ethanol, 2% B-27 in Neurobasal) and plated on glass coverslips precoated with poly-L-lysine. Under these conditions, glial cell growth was maintained at less than 0.5% for a nearly pure neuronal population.

Synthetic $A\beta_{42}$ (American Peptide, Sunnyvale, CA) was resuspended in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), sonicated, and stored at −80 °C. Preparations were thawed, dried under nitrogen gas, and dissolved in 0.04 M Tris, 0.3 M NaCl (pH 7.4) at 120 μM. Aβ was applied onto neuronal cultures grown for 4 days in vitro. Cells were incubated for 72 h with 5 μM $A\beta_{42}$ in the presence or absence of compounds.

Using the DNA-binding dye Hoechst 33342 (Molecular Probes, Burlington, ON), cell death was assessed by detecting condensed or fragmented chromatin. The coverslips covered with primary neurons were stained with Hoechst 33342 (2 μg/ml) for 10 min, fixed with ice-cold methanol for 5 min, washed in PBS, and mounted onto glass slides using ProLong anti-fade reagent (Molecular Probes). Nuclei were analyzed at 200× magnification using fluorescence microscopy. Live cells and morphologically dead cells were scored from five randomly selected fields for each condition ($N \geq 80$ cells per condition). The data are expressed as a percentage of toxicity ([number of dead cells/total number of cells] × 100).