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Trichostatin A increases the levels of plasma gelsolin and amyloid beta-protein in a transgenic mouse model of Alzheimer's disease

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ABSTRACT

Aims: Gelsolin (GSN), a multifunctional protein, binds to amyloid beta-protein (Aβ), inhibits its fibrillization, solubilizes preformed Aβ fibrils, and helps in its clearance from the brain. Trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, induces the protein expression of gelsolin. In the present study, we investigated how TSA-treatment of APPswe/PS1$_{\delta E9}$ transgenic (Tg) mice of Alzheimer's disease (AD) will affect the plasma levels of gelsolin and Aβ.

Main methods: TSA (5 mg/kg body weight on alternate days for two months) was intraperitoneally injected to AD Tg mice. Gelsolin was measured by Western blotting and Aβ was measured by enzyme-linked immunosorbent assay.

Key findings: TSA-treatment significantly increased the levels of plasma gelsolin by 1.79-fold as compared with vehicle-treated control mice ($p < 0.01$). The levels of Aβ 1–40 and Aβ 1–42 in the plasma were also higher in TSA-treated mice in comparison with vehicle-treated mice. The treatment of transgenic AD mice with TSA did not affect the body weight in both male and female groups as compared to vehicle-treated animals. A positive correlation was observed between the plasma levels of gelsolin and Aβ 1–40 ($r = 0.594$, $p = 0.042$) or Aβ 1–42 ($r = 0.616$, $p = 0.033$) in AD Tg mice.

Significance: These results suggest that TSA increases the levels of plasma gelsolin and Aβ in AD Tg mice, which may have implications in gelsolin-mediated clearance of Aβ.

Introduction

Gelsolin (GSN), a multifunctional 90 kDa protein, is a major actin-binding protein. It plays important roles in various diseases such as cancer, Alzheimer's disease (AD), pulmonary disease, cardiac injury, infections, and inflammation as well as in apoptosis (Li et al., 2012). It is present intracellularly as a cytoplasmic protein, and in the biological fluids, i.e., plasma/cerebrospinal fluid (CSF) as a secreted protein (Kwiatkowski et al., 1988). Both forms of gelsolin are products of alternative splicing of a single 70 kbp-long gene. However, they differ in the length and the presence of disulfide groups. Plasma gelsolin has a 25 amino acid-signal peptide at its amino-terminus (Yin et al., 1984). There are five cysteine (Cys) residues in gelsolin. All five Cys residues in cytoplasmic gelsolin are free thiols; whereas in plasma gelsolin, three Cys residues are free thiols and the other two are disulfide-linked (Wen et al., 1996).

Extracellular deposition of fibrillar amyloid beta-protein (Aβ) as amyloid plaque, and intracellular formation of neurofibrillary tangles due to hyperphosphorylation of tau are two hallmarks of AD (Glenner, 1983; Huang and Jiang, 2009). Aβ is formed by proteolytic cleavage of amyloid precursor protein (APP) (Pluta et al., 2013). Aβ is normally present as a soluble protein. However, in pathological conditions such as AD, it gets fibrillized and deposited as amyloid plaque in the brain. Fibrillar Aβ 1–40 and Aβ 1–42 are two major constituents of amyloid plaques in AD. Various attempts have been made to prevent the fibrillization of Aβ in both human and transgenic (Tg) animal model of AD (Antequera et al., 2009; Lahiri et al., 2005; Matsuoka et al., 2003). Since our initial reports on binding of gelsolin to Aβ (Chauhan et al., 1999; Ji et al., 2008, 2010) and inhibition of Aβ fibrillization as well as solubilization of Aβ fibrils by gelsolin (Ray et al., 2000), several studies have confirmed anti-amyloidogenic role of gelsolin in transgenic animal models of AD where it could reduce amyloid load (Antequera et al., 2009; Hirko et al., 2007; Lahiri et al., 2005; Matsuoka et al., 2003). Matsuoka et al. (2003) reported that peripheral administration of plasma gelsolin could reduce the amyloid load in mutant APP-Tg mice. Similarly, Hirko et al. (2007) showed that transgene expression of plasma gelsolin in APP/PS1$_{\delta E9}$ mice decreased amyloid load. In another study, virally directed expression of gelsolin also reduced the amyloid load in double-transgenic APP/PS1 mice (Antequera et al., 2009). In a recent study, peripheral administration of gelsolin reduced the cerebral amyloid angiopathy (accumulation of Aβ in the walls of leptomeningeal and cortical blood vessels of brains) in Tg2576 mice model of AD (Gregory et al., 2012).

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The levels of gelsolin can be increased epigenetically by inhibition of histone deacetylase. Histone deacetylase inhibitors such as sodium butyrate (Kamitani et al., 2002) and trichostatin A (TSA) (Hoshikawa et al., 1994; Kamitani et al., 2002; Yildirim et al., 2008) have been reported to increase the expression of gelsolin in cell cultures and brain. However, a relationship between TSA-induced gelsolin and Aβ in transgenic AD mice has not been studied. Therefore, we studied whether TSA can be used as a potential therapeutic agent in AD for clearance of Aβ by increasing the levels of plasma gelsolin in APPswe/$PS1_{\delta E9}$ transgenic mice. We report here that TSA increases the levels of gelsolin, Aβ 1–40 and Aβ 1–42 in the plasma. The levels of plasma gelsolin correlated with the levels of plasma Aβ 1–40 and Aβ 1–42, suggesting that plasma gelsolin probably acts as "peripheral sink protein" to bind Aβ peptides, and may help in Aβ clearance from the brain or other tissues.

Materials and methods

Animal treatment

Twenty six APPswe/$PS1_{\delta E9}$ transgenic mice (13 males and 13 females) at the age of 4 months were purchased from Jackson Laboratory (Bar Harbor, ME) and kept in the animal colony with the food and water *ad libitum*. All instructions were followed according to the National Institutes of Health Guidelines for the Humane Treatment of Animals, and the protocol was approved by the Animal Welfare Committee of NYS Institute for Basic Research in Developmental Disabilities.

The mice were divided randomly into two treatment groups: TSA (7 females, 7 males) and vehicle control (6 females, 6 males). The intraperitoneal injections of TSA or vehicle to the mice were started at the age of 9 months, and continued for 60 days.

Five milligrams of TSA (Selleck Chemicals, Houston, TX) was dissolved in 0.1 ml of dimethyl sulfoxide (DMSO), and then diluted with 9.9 ml of phosphate buffered saline (PBS). The mice were intraperitoneally injected with TSA at a dose of 0.1 ml/10 g (i.e., 5 mg/kg body weight) every alternate day for a total of 30 treatments. The injection spot was alternated between the left and right sides to avoid peritonitis. The control mice were injected with the same dose of vehicle (no TSA, i.e., DMSO diluted in PBS). The body weights of mice were monitored every week, and the dose of TSA was calculated on the basis of weekly body weight.

After 30 treatments, the mice were anesthetized with ether and sacrificed. The blood samples were withdrawn and transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. After centrifugation at 1000 *g* for 10 min at 4 °C, the plasma samples were collected and stored at −80 °C for further use.

Western blotting for gelsolin

The protein concentrations of plasma samples were measured with the BCA (bicinchoninic acid) Protein Assay Kit (Thermo Scientific, Rockford, IL). The plasma samples were diluted ten-fold with PBS. The samples were mixed with loading buffer, and boiled for 5 min. The denatured plasma proteins were separated on a 10% SDS-PAGE gel, and then transferred to a nitrocellulose membrane (0.45 μm; Bio-Rad Laboratories, Hercules, CA). After blocking with 5% non-fat milk for 1 h at room temperature (RT), the membrane was incubated overnight at 4 °C with 5% non-fat milk containing rabbit monoclonal anti-gelsolin antibody (1:25,000; Abcam, Cambridge, MA). After 3 washes with Tris-buffered saline-0.05% Tween-20 (TBST), the membrane was further incubated with the horseradish peroxidase-conjugated secondary antibody (1:5000; Thermo Scientific, Rockford, IL) for 1 h at RT. The membrane was again washed 3 times, and the immunoreactive proteins were visualized using the ECL substrate (Thermo Scientific, Rockford, IL). The densities of the gelsolin bands were measured using ImageJ software (NIH, Bethesda, MD). The data was normalized with total protein content of the samples.

Measurement of plasma Aβ 1–40 and Aβ 1–42 levels by sandwich enzyme-linked immunosorbent assay [ELISA]

The levels of Aβ 1–40 and Aβ 1–42 in the plasma samples of TSA- and vehicle-treated AD Tg mice were measured by ELISA as described previously but with minor modifications (Mehta et al., 2000). Briefly, 100 μl of monoclonal Aβ antibody 6E10 (2.5 μg/ml) diluted in carbonate–bicarbonate buffer (pH 9.6) was coated in the wells of microtiter plate, and allowed to incubate overnight at 4 °C. After washing the plates with PBS containing 0.05% Tween 20 (PBST), the wells were blocked for 1 h with 200 μl of 10% normal sheep serum in PBS to avoid nonspecific binding. The plates were again washed, and 100 μl of standards (Aβ 1–40 and Aβ 1–42; Bachem, Torrance, CA) diluted in PBST with 0.5% bovine serum albumin (BSA) or plasma samples (diluted 1:2) were added and incubated for 2 h at RT, followed by overnight incubation at 4 °C. After washing, the plates were incubated with rabbit monoclonal antibody (clone 5-139) specific to a peptide corresponding to Aβ 1–40 or rabbit monoclonal antibody (clone 1-11-4) specific to a peptide corresponding to Aβ 1–42 diluted in PBST with 0.5% BSA at RT for 2 h. After washing, goat anti-rabbit IgG peroxidase (Invitrogen, Grand Island, NY) diluted in PBST was added to the wells, and the plates were incubated for 2 h at RT. The plates were again washed, and QuantaBlu flurogenic peroxidase substrate (Thermoscientific, Rockland, IL) was added. The reaction was stopped after 30 min by adding QuataBlu stop solution. The relative fluorescence units (RFU) for each well were measured according to the kit instructions. The relationship between RFU and the Aβ 1–40 or Aβ 1–42 peptide concentrations was determined using a 4-parameter logistic logarithm function.

Statistical analysis

Data is presented as Mean ± SEM. The unpaired Student's *t*-test was used to compare the data between the TSA-treated group and vehicle-treated control group by using GraphPad prism 5.0 (GraphPad Software, Inc., La Jolla, CA). Linear regression analysis was done to study the correlation between the levels of plasma gelsolin and Aβ 1–40/Aβ 1–42, and the Pearson correlation coefficient (r) and *p* values were obtained. In addition, 95% confidence interval (CI) of vehicle-treated control group was used as the normal range to evaluate whether the data of the TSA-treated group were out of the normal range. The *p* values less than 0.05 were considered as the significant difference.

Results

Effect of TSA-treatment on the body weight of mice

The body weights of TSA-treated and vehicle-treated control AD Tg mice were measured every week. Percent increases in body weight of TSA-treated or vehicle-treated control mice (males or females) are shown in Fig. 1. There was no effect of TSA-treatment for two months (9th to 11th months of age) on the body weight of either female (Fig. 1A) or male Tg mice (Fig. 1B) as compared to vehicle-treated control Tg mice.

TSA-treatment increases levels of gelsolin in the plasma of AD Tg mice

Fig. 2A shows the Western blot of plasma gelsolin of TSA-treated and vehicle-treated AD Tg mice. The density of the gelsolin bands was normalized by the total protein content of the samples. TSA-treatment increased the plasma concentration of gelsolin by 82% in female (39.98 ± 8.41/μg protein, n = 7) (Fig. 2B) and by 76% in male mice (45.68 ± 5.98/μg protein, n = 7) (Fig. 2C) as compared to vehicle-treated control mice (female group, 21.91 ± 4.24/μg protein, n = 6, and male group, 26.01 ± 3.72/μg protein, n = 6). A significant difference