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Ceftriaxone ameliorates tau pathology and cognitive decline via restoration of glial glutamate transporter in a mouse model of Alzheimer's disease

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ABSTRACT

Glial glutamate transporter, GLT-1, is the major Na^+-driven glutamate transporter to control glutamate levels in synapses and prevent glutamate-induced excitotoxicity implicated in neurodegenerative disorders including Alzheimer's disease (AD). Significant functional loss of GLT-1 has been reported to correlate well with synaptic degeneration and severity of cognitive impairment among AD patients, yet the underlying molecular mechanism and its pathological consequence in AD are not well understood. Here, we find the temporal decrease in GLT-1 levels in the hippocampus of the 3xTg-AD mouse model and that the pharmacological upregulation of GLT-1 significantly ameliorates the age-dependent pathological tau accumulation, restores synaptic proteins, and rescues cognitive decline with minimal effects on Aβ pathology. In primary neuron and astrocyte coculture, naturally secreted Aβ species significantly downregulate GLT-1 steady-state and expression levels. Taken together, our data strongly suggest that GLT-1 restoration is neuroprotective and Aβ-induced astrocyte dysfunction represented by a functional loss of GLT-1 may serve as one of the major pathological links between Aβ and tau pathology.

1. Introduction

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease associated with dementia. Neuropathological hallmarks include extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) composed of Aβ peptides and hyperphosphorylated tau proteins, respectively. Buildup of these pathological lesions are believed to trigger complex, multifactorial neurodegenerative cascades in AD leading to synaptic loss and neurodegeneration. Dementia severity among AD patients correlates well with synaptic loss and oligomeric Aβ species and to a lesser extent, with Aβ plaques in the brain (Dickson et al., 1992; Haass and Selkoe, 2007; Hardy and Selkoe, 2002; Scheff et al., 2006; Selkoe, 2002; Tomic et al., 2009). Moreover, increasing Aβ levels has been suggested to be an initiating factor in AD pathology (Haass and Selkoe, 2007). The exact molecular mechanism that links Aβ and tau pathology in AD, however, remains largely unknown.

Clinical and preclinical evidence suggests disruption of glutamate homeostasis contributes to the development of neuropathological hallmarks and cognitive decline in AD. Glutamate neurotransmission is critically involved in learning and memory, and its synapses are densely concentrated in the hippocampus, a vulnerable region affected in AD. Its neurotransmission is tightly controlled by 5 different glutamate transporters within the vicinity of glutamatergic synapses in humans to prevent prolonged glutamate input and subsequent glutamate-induced excitotoxicity in neurons. Among these transporters, the excitatory amino acid transporter 2 or its mouse homologue glutamate transporter 1 (GLT-1, herein collectively referred to as GLT-1) expressed predominantly on astrocytes is responsible for regulating 90% of glutamate levels in the synapses (Kim et al., 2011). A significant reduction of GLT-1 activity has been reported to occur in an early stage of AD and correlates well with synaptic loss and cognitive decline in patients (Masliah et al., 1996). In addition, GLT-1 gene expression and protein levels are altered in the hippocampus of AD patients (Jacob et al., 2007). Recent studies further support the

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involvement of GLT-1 in AD by showing that the heterozygous knockdown of GLT-1 in an AD mouse model exacerbates cognitive decline without affecting Aβ pathology and that astrocytic GLT-1 dysfunction plays an important role in human AD pathogenesis (Mookherjee et al., 2011; Woltjer et al., 2010). These studies evidently show that impairment in GLT-1 is not only part of the initial stages of AD pathology but that it also plays an important role in the progression of cognitive decline.

In this study, we investigate whether compensation for the age- and pathology-dependent loss of GLT-1 would significantly impact AD-like neuropathology and cognitive decline in the triple transgenic mouse model of AD (3xTg-AD). We hypothesize that a loss of GLT-1, representative of functional impairments of astrocytes, mediates Aβ-induced neurotoxicity and precedes postsynaptic degeneration and cognitive decline in AD, and its restoration rescues functional synapses and halts the disease progression. Here, we show that the chronic upregulation of GLT-1 by ceftriaxone significantly attenuates tau pathology, restores synaptic proteins, and rescues cognition without affecting Aβ pathology in 3xTg-AD mice. Our in vitro studies uncover that naturally secreted Aβ species significantly downregulate GLT-1 expression. Taken together, loss of GLT-1 may in part mediate Aβ-triggered tau pathology in AD.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of California and were consistent with Federal guidelines. All mice were housed on a 12-hour light-dark schedule with ad libitum access to food and water. The 3xTg-AD mice express the Swedish (K670N/M671L), $PS1_{M146V}$, and tau (P301L; found in frontotemporal dementia patients) mutations which increase the overall production of Aβ, increases the ratio of $Aβ_{42}/Aβ_{40}$ and promotes tau tangle formation, respectively (Oddo et al., 2003b). A total of 5–7 3xTg-AD mice and 5–7 strain-matched C57BL6/129SvJ nontransgenic mice were used for the aging study. A total of 15 3xTg-AD mice were treated; 4 females and 4 males for the vehicle group and 3 females and 4 males for the ceftriaxone-treated group.

2.2. Animal treatment paradigm

Ten-month-old 3xTg-AD mice were treated intraperitoneally (i.p.) with 200 mg/kg ceftriaxone (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or 0.8% sodium chloride (Vehicle) daily for 2 months (Rothstein et al., 2005). On completion of the treatment period and cognitive evaluation, mice were anesthetized and perfused with ice-cold phosphate-buffered saline and brains were collected. One hemisphere of the brain was fixed with 4% paraformaldehyde for immunostaining. The other hemisphere was microdissected into the hippocampus and cortex. The hippocampus was homogenized in T-PER buffer containing protease and phosphatase inhibitors followed by the centrifugation at 100,000*g* for 1 hour to separate the detergent-soluble fraction and insoluble pellets. Pellets were subsequently homogenized in 70% formic acid and the detergent-insoluble fractions were collected. Samples were stored at −80 °C until further analysis.

2.3. Cognitive tests

After the 2 months of ceftriaxone treatment, all mice were subjected to cognitive evaluation in the Morris water maze (MWM) and novel object recognition test (NOR). As described previously (Kitazawa et al., 2011), the apparatus used for the MWM task was a circular aluminum tank (1.2-m diameter) painted white and filled with water maintained at 22–24 °C. The maze was located in a room containing several simple visual extramaze cues. Mice were trained to swim and find a 14-cm diameter circular clear Plexiglas platform submerged 1.5 cm beneath the surface of the water and invisible to the mice while swimming. On each training trial, mice were placed into the tank at 1 of 4 designated start points in a pseudorandom order. Mice were allowed to find and escape onto the platform. If mice failed to find the platform within 60 seconds, they were manually guided to the platform and allowed to remain there for 10 seconds. Each day, mice received 4 training sessions separated by intervals of 25 seconds under a warming lamp. The training period ended when all groups of mice reached criterion (<25 seconds mean escape latency). The probe trial to examine retention memory was assessed 24 hours after the last training trial. In the probe trials, the platform was removed from the pool, and mice were monitored by a ceiling-mounted camera directly above the pool during the 1-minute period. All trials were recorded for subsequent analysis. The parameters measured during the probe trial included: (1) latency to cross the platform location and (2) number of platform location crosses.

The NOR was performed as described previously (Blurton-Jones et al., 2009). Briefly, mice were habituated in the test environment for 3 days (5 minutes per day). In the acquisition of familiar objects, mice were exposed to 2 identical objects separated in a specific location in a square cage. Twenty-four hours later, mice were placed in the test cage with 1 familiar object and 1 novel object. The total amount of time the mice explored each object was recorded separately for 3 minutes, and the recognition index ([time spent on novel object]/[{time to spent on novel object} + {time spent on a familiar object}]) was calculated in %.

2.4. Quantitative Aβ enzyme-linked immunosorbent assay analysis

Soluble and insoluble $Aβ_{1\text{-}40\ and}$ $Aβ_{1\text{-}42}$ levels were measured by enzyme-linked immunosorbent assay (ELISA) as previously described (Kitazawa et al., 2011). Briefly, 96-well plates (Immulon 2HB, Fisher Scientific, Waltham, MA, USA) were coated with 25 μg/mL of the mouse anti-Aβ monoclonal antibody (clone 20.1) in carbonate coating buffer pH 9.6 (Sigma-Aldrich, St. Louis, MO, USA) and incubated overnight at 4 °C. The wells were washed and blocked with 3% bovine serum albumin (BSA) overnight at 4 °C with shaking. After washing, serial dilutions of $Aβ_{40}$ and $Aβ_{42}$ were added to the wells and plates were sealed then incubated overnight at 4 °C with shaking. After washing, horseradish peroxidase–conjugated affinity anti-$Aβ_{40}$ or anti-$Aβ_{42}$ antibodies were added at 1:2000 and 1:1000 dilutions, respectively, and incubated overnight at 4 °C with shaking. Wells were then washed and incubated with streptavidin–horseradish peroxidase (1:4000 dilution) for 4 hours at room temperature, washed then Ultra-TMB ELISA substrate (Pierce, Rockford, IL, USA) was added for 5–10 minutes to develop the reaction. The reaction was stopped by adding 2N H_2PO_4 and plates were analyzed on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. The plasma end-point titer was defined as the maximal plasma dilution in which the optical density for the antibodies was 3 times higher than the optical density values of the blank wells.

2.5. Western blot analysis

Protein concentrations of detergent-soluble fractions from half brain (hippocampus or cortex) were determined by the Bradford protein assay. These fractions (9 μg of protein) were subsequently immunoblotted with the following antibodies: HT7 (total human tau; Pierce Biotechnology), AT8 (phosphorylated tau at S202/T205; Pierce Biotechnology), PHF-1 (phosphorylated tau at S396/S404; a