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Intranasal telmisartan ameliorates brain pathology in five familial Alzheimer's disease mice

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ABSTRACT

The renin-angiotensin system (RAS) is a major circulative system engaged in homeostasis modulation. Angiotensin II (Ang II) serves as its main effector hormone upon binding to its primary receptor, Ang II receptor type 1 (AT_1R). It is well established that an intrinsic independent brain RAS exists. Abnormal AT_1R activation both in the periphery and in the brain probably contributes to the development of Alzheimer's disease (AD) pathology that is characterized, among others, by brain inflammation. Moreover, treatment with drugs that block AT_1R (AT_1R blockers, ARBs) ameliorates most of the clinical risk factors leading to AD.

Previously we showed that short period of intranasal treatment with telmisartan (a brain penetrating ARB) reduced brain inflammation and ameliorated amyloid burden (a component of Alzheimer's plaques) in AD transgenic mouse model. In the present study, we aimed to examine the long-term effect of intranasally administrated telmisartan on brain inflammation features including microglial activation, astrogliosis, neuronal loss and hippocampus-dependent cognition in five-familial AD mouse model (5XFAD). Five month of intranasal treatment with telmisartan significantly reduced amyloid burden in the cortex and hippocampus of 5XFAD mice as compared with the vehicle-treated 5XFAD group. Similar effects were also observed for CD11b staining, which is a marker for microglial accumulation. Telmisartan also significantly reduced astrogliosis and neuronal loss in the cortex of 5XFAD mice compared with the vehicle-treated group. Improved spatial acquisition of the 5XFAD mice following long-term intranasal administration of telmisartan was also observed. Taken together, our data suggest a significant role for AT1R blockage in mediating neuronal loss and cognitive behavior, possibly through regulation of amyloid burden and glial inflammation.

1. Introduction

The renin-angiotensin system (RAS) is a major circulative system in homeostasis modulation (Skrbic and Igic, 2009). Angiotensinogen is converted by renin to form angiotensin I (Ang I), which in turn is converted into the active angiotensin II (Ang II) hormone (Wright and Harding, 2013). The Ang II receptor type 1 (AT_1R) is the primary receptor for Ang II (Saavedra, 2012).

It is well established that an intrinsic independent brain RAS exists and expresses all of its components in the central nervous system (CNS) (Wright et al., 2008). RAS receptors have been observed in several brain areas including the hippocampus and the cingulate cortex (McKinley and et al., 2003). Brain RAS was suggested to be involved in multiple regulatory functions such as emotional responses, inflammation and memory consolidation (Haulica and et al., 1999; Llorens-Cortes and Mendelsohn, 2002). AT_1R activation in the brain contributes to the control of cerebral blood flow, central sympathetic activity, blood- brain barrier (BBB) integrity and the regulation of immune responses (Saavedra, 2016). Over-activation of brain AT_1R is associated with pathological processes including brain inflammation, breakage of the BBB, and cognitive decline (Saavedra, 2012; Nishimura et al., 2000). In fact, abnormal AT_1R activation both in the periphery and brain may contribute to the development of Alzheimer's disease (AD) pathology characterized by brain dysfunction and cognitive decline (Saavedra, 2016).

Glial cells activation, brain inflammation and oxidative stress were demonstrated to lead to cell dysfunction and participate in a self- propagating cycle of neurodegeneration (Aisen, 2002;

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Lyman and et al., 2014). During brain inflammation, astrocytes and microglia become reactive and overproduce pro-inflammatory cytokines and chemokines into the brain parenchyma (Kettenmann and et al., 2011; Li and et al., 2011). In AD, accumulation of reactive astrocytes and microglia were reported to be in close proximity to extracellular amyloid β (Aβ) depositions, a well-known neuropathological hallmark of the disease (Doens and Fernandez, 2014; Akiyama and et al., 2000). Aβ plaques may also provoke brain inflammation and play a pivotal role in the vicious cycle ascribed to the disease (Aisen, 2002). During the course of brain inflammation, glial activation is mainly attributed to neurotoxicity and cell death (Heneka and et al., 2010; Spangenberg and Green, 2017). However, these cells may also have neuroprotective roles by releasing trophic factors and expressing a phagocytic phenotype, which results in Aβ clearance (Doens and Fernandez, 2014; Gebicke-Haerter, 2001). Microglia are likely to exist in a range of phenotypic states during chronic inflammation in AD (Martinez et al., 2009; Colton, 2009; Colton and et al., 2006; ElAli and Rivest, 2016).

In vitro studies have shown that blockage of the AT_1R stimulation by Ang II AT_1 receptor blockers (ARBs) not only reduced the pro- inflammatory mediators production by astrocytes and microglial cells (Lanz and et al., 2010; Benicky and et al., 2011; Miyoshi and et al., 2008), but also shifted microglial activation state to the neuroprotective phenotype (Xu and et al., 2015). ARBs are clinically used as cardiovascular and metabolic disorders treatment (Aulakh et al., 2007). Moreover, treatment with ARBs ameliorates most of the clinical risk factors leading to AD, including hypertension and diabetes. (Saavedra, 2016, 2012). Telmisartan, an ARBs family member, was reported to cross the BBB when administered systematically (Saavedra, 2016). A previous study conducted on primary human neuroblasts and rat cortical neurons cells showed that telmisartan ameliorated interleukin 1β (IL1β)-induced oxidative stress and inflammation (Pang and et al., 2012). However, the effect of telmisartan on neuronal loss associated with brain inflammation was not investigated in AD mouse model, let alone by intranasal administration.

We previously reported that a short period of intranasal treatment with telmisartan reduced glial inflammation and ameliorated amyloid burden and microglial activation in AD transgenic mouse model (Torika et al., 2016). In the present study, we examined the long-term effect of intranasally administrated telmisartan on brain inflammation features including microglial activation, astrogliosis, neuronal loss and hippocampus-dependent cognition in five familial AD mouse model (5XFAD).

2. Materials and methods

2.1. Mice

5XFAD mice model that harbor three familial Alzheimer's disease (FAD) mutations in the human APP695 (Swedish K670N, M671L; Florida I716V and London V717I) and two mutations in the human presenilin-1 (PSEN-1) (M146L, L286V) gene under the transcriptional control of the neuron-specific mouse Thy-1 promoter, were used. Hemizygous 5XFAD transgenic mice were reproduced with C57BL/6 wild type (WT) mice purchased from Harlan (Jerusalem, Israel). Mice were placed in cages under temperature (22 ± 2 °C) and humidity (65 ± 5%) controlled conditions with a 12 h light/dark cycle. Food and water supply was available. Three weeks neonatal mice underwent DNA tail genotyping analysis for the human APP gene and marked as WT or 5XFAD. Male and female mice were arbitrarily divided into three different groups as follows: (1) 1 mg/kg/day telmisartan (Tocris Bioscience, Bristol UK)- treated WT mice (WT + Tel) used as a control group (n = 8 mice; 3 female/5 male), (2) 1 mg/kg/day telmisartan-treated 5XFAD mice (5XFAD + Tel) (n = 9; 4 female/5 male) and (3) vehicle-treated 5XFAD mice (5XFAD + vehicle) (n = 8; 3 female/5 male). Telmisartan dissolved in N,N-dimethylformamide/polyethy lene glycol 400/saline (2:6:2) solvent at concentration of 2 mg/ml (Noda and et al., 2012). Mice were placed in a supine position and administered with the indicated solutions for 5 days/week using pipettor, as 3 μl drop to each nostril. Five-month intranasal treatment procedure began as the mice reached eight weeks old. All experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee of Ben-Gurion University of the Negev (Beer Sheva, Israel; approval number IL-30-08-2011-15).

2.2. Protein lysate preparation and Western blot

Mice were anesthetized with intra peritoneal (i.p) injection of 0.1 ml ketamine hydrochloride and 0.1 ml xylazine hydrochloride and cardiac perfusion was performed using cold phosphate-buffered saline (PBS). Hippocampal and cortical areas were removed from one hemisphere and immediately frozen in liquid nitrogen followed by -80 °C storage. For protein extraction, tissues were weighted and homogenized on ice lysis buffer containing phosphatase and protease inhibitors (Sigma–Aldrich), rotated on ice for 30 min and centrifuged for another 30 min in 14,000g 4 °C. Supernatants were collected and protein concentrations were measured using the Bio-Rad protein assay (Bio-Rad Laboratories, USA). Equal amounts of protein from tissue lysates were subjected to 7.5% or 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. 4% bovine serum albumin (BSA) was used for membrane blocking and membranes were incubated overnight at 4 °C with one of the following antibodies: Rabbit anti-inducible nitric oxide synthase (iNOS) antibody (1:500, Cayman Chemicals, USA), rabbit anti-arginase-1 (ARG-1) antibody (1:200, Santa Cruz Biotechnology, Inc., USA), rabbit anti-CD10/Neprilysin (NEP) antibody (1:4000, Abcam, UK), rabbit anti-insulin degrading enzyme (IDE) antibody (1:4000, Calbiochem, Merck Millipore, USA), mouse anti-Glial fibrillary acidic protein (GFAP) antibody (1:1000, Calbiochem, Merck Millipore, USA), mouse anti-Neuronal Nuclei (NeuN) antibody (1:750, Calbiochem, Merck Millipore, USA) and mouse anti-β-actin antibody (1:4000, Sigma-Aldrich) that was used for protein load normalization. Thereafter, membranes were washed and incubated with the corresponding-conjugated antibody at room temperature for 90 min. The following antibodies were used: Donkey anti-rabbit antibody (1:10000, GE Healthcare, Buckinghamshire, UK) and horseradish peroxidase-conjugated goat anti-mouse antibody (1:20,000, Jackson immunoResearch Inc., USA). Proteins position was detected using enhanced chemiluminescence (ECL) solution and exposure to X-ray film (Fuji medical X-ray film, FujiFilm). Bands intensity was quantified using a computerized image analysis system (EZ Quant-Gel 2.2, EZQuant Biology Software Solutions Ltd., Israel).

2.3. Immunohistochemistry

Mice were anesthetized and cardiac perfusion was performed as previously described. Mice brains were then removed and the hemispheres were immediately incubated overnight in cold 4% paraformaldehyde (PFA) solution (4 °C). Thereafter, the brains were transferred into 30% sucrose solution for another 48 h in 4 °C and frozen in molds containing tissue adhesive (O.C.T compound, Tissue-Tek, Torrance, CA, USA) at −80 °C. 40 μm thick sagittal slices (sliced by cryostat, Leica, Germany) were rinsed in 0.05% PBS/Tween 20 and 0.5% PBS/Triton X-100 solutions. Then, blocking was performed using primary antibody diluting buffer (GBI labs,