Telmisartan Treatment Ameliorates Memory Deficits in Streptozotocin-Induced Diabetic Mice via Attenuating Cerebral Amyloidosis

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Abstract. Telmisartan, an angiotensin II type 1–receptor blocker (ARBs), has been reported to exert beneficial effects on the central nervous system (CNS). However, the effect of telmisartan on cognitive impairment associated with type 1 diabetes is not well known. Here, we examined the possibility that telmisartan could improve memory function in a type 1 diabetic mouse model, streptozotocin (STZ)-induced diabetic mice. STZ-induced diabetic mice subjected to the Morris Water Maze (MWM) task exhibited a significant decline of spatial learning and memory. Oral administration of telmisartan at two nonhypotensive doses (0.7 or 0.35 mg/kg) significantly improved memory deficits in STZ-induced diabetic mice. Telmisartan treatment markedly reduced Aβ42, APP, BACE1, RAGE, and NF-κB p65 of the hippocampus and cortex, but did not beneficially affect hyperglycemia and hypoinsulinemia in the STZ-induced diabetic mice compared with untreated diabetic mice. Taken together, our findings suggest that telmisartan ameliorates memory deficits in type 1 diabetic mice, at least partly because of attenuation of amyloidosis in the brain.

Keywords: type 1 diabetes, telmisartan, memory, amyloidosis

Introduction

Diabetes has negative impacts on the central nervous system leading to diabetic encephalopathy and concomitant augmented incidence of cognitive problems (1, 2). Recently, it has been suggested that both type 1 and 2 diabetes play a significant role in brain diseases such as Alzheimer’s disease (3, 4). The impairment of cognitive capacities is reportedly associated with the increasing amount of amyloid plaques and neurofibrillary tangles in the hippocampus at autopsy in diabetes (5). Amyloid-β (Aβ) accumulation also occurred in the brain of diabetic animals spontaneously (6) or induced by streptozotocin (STZ), a diabetogenic agent, toxic to β cells of pancreatic islands (7, 8). Furthermore, diabetes-accelerated memory dysfunction was due to cerebrovascular inflammation and Aβ deposition in an Alzheimer’s disease mouse model with diabetes (9). These findings suggested that Aβ accumulation in the brain in the diabetic condition may be one of the important causes for diabetes-associated cognition impairment.

Telmisartan, an angiotensin II type 1–receptor (AT1R) blocker (ARB), is widely used in patients with hypertension with the expectation of a decrease in the onset of cardiovascular and cerebrovascular disease. Recently, the focus on ARBs has been intensified because their novel biological roles have emerged, particularly for their therapeutic potential in brain disorders (10, 11). It has been reported that telmisartan has a beneficial effect in a murine model of ischemia/reperfusion injury through blockade of AT1R and exhibited neural protection, including anti-apoptosis, anti-inflammatory, and anti-oxidant benefits in the intracerebral hemorrhage rat model (12, 13). Recent studies showed that telmisartan protects mouse dopaminergic neurons and inhibits the microglial response in a mouse MPTP model of Parkinson’s disease (14). Furthermore, treatment of telmisartan significantly...
attenuated hypertension-induced learning and memory deficits, endothelial dysfunction, and changes in various biochemical parameters (15, 16). In some experimental animal models of Alzheimer’s disease, telmisartan exhibited significant ameliorative effects on the impaired spatial memory and inflammatory response (17 – 20). However, to date, less attention has been given to the effect of telmisartan on the diabetes-associated cognitive impairment. Thus, we used streptozotocin (STZ)-induced diabetic mice similar to those with type 1 diabetes to observe the effects of telmisartan on memory deficits and cerebral amyloidogenesis in this study.

Materials and Methods

Materials
Telmisartan was purchased from Jiangsu Hengrui Medicine Co., Ltd. (Nanjing, China). Streptozotocin (STZ) was purchased from Sigma (St. Louis, MO, USA). Antibodies were purchased from different companies: anti-Aβ42 and anti-receptor for advanced glycation end products (RAGE) from Abcam Technology Co., Ltd. (Hongkong, China); anti-β-amyloid precursor protein (APP) and anti-β-site APP cleaving enzyme 1 (BACE1) and anti-nuclear factor κB (NF-κB) p65 from Cell Signaling Technology (Boston, MA, USA). Anti-β-actin and secondary antibodies were obtained from Bioworld Technology Co., Ltd. (Minneapolis, MN, USA). Glucose Oxidase Kit and insulin Sandwich ELISA kit were purchased from Nanjing Jiancheng Biotech Institute (Nanjing, China), and all other chemicals were of analytical grade and commercially available.

Animals
ICR male mice (8 – 10-week-old, weighing 20 – 25 g) were purchased from Medical Center of Yangzhou University (Yangzhou, China). All animal procedures were performed in accordance with the guidelines of the institutional animal care and use committee of China. Mice were housed eight per cage and allowed access to diet and autoclaved water. Animal housing rooms were maintained at a constant room temperature (25°C) in a 12-h light (7:00 A.M.) / dark (7:00 P.M.) cycle.

Generation of diabetic model and treatment with telmisartan
Mice were intravenously injected once with STZ (150 mg/kg) to induce experimental type 1 diabetes characterized by hyperglycemia. Animals with similar degrees of hyperglycemia and body weight were randomly divided into 3 groups, STZ plus vehicle group (STZ + Veh), STZ plus 0.70 mg/kg telmisartan group (STZ + Tel 0.70 mg/kg), and STZ plus 0.35 mg/kg telmisartan group (STZ + Tel 0.35 mg/kg). Age-matched control mice were injected with the vehicle and divided into 3 groups: vehicle plus vehicle group (Veh + Veh), vehicle plus 0.70 mg/kg telmisartan group (Veh + Tel 0.70 mg/kg), and vehicle plus 0.35 mg/kg telmisartan group (Veh + Tel 0.35 mg/kg). After grouping, diabetic mice were intragastrically administered with telmisartan dissolved in 0.5% sodium carboxymethyl cellulose (CMC-Na). After 5 weeks of consecutive treatment (i.e., once daily), some of the mice were submitted to the Morris water maze (MWM) task for 6 days; the remaining mice were used for testing blood pressure and determining blood glucose or insulin, and the brain tissues were taken out for assays of Aβ42, APP, BACE1, RAGE, and NF-κB p65 in the hippocampus and cortex.

Blood pressure measurement
Mice were anesthetized with the intraperitoneal injection of 350 mg/kg chloral hydrate and then immobilized on the operation table for 1 h after the last administration. The skin along midline was cut, and then the right carotid artery was isolated and inserted with an arterial cannula filled with heparinized saline and connected to force-displacement transducer linking the physiological pressure detector. Mean arterial pressure was recorded and analyzed with the use of a PC-based data acquisition system (BL420F, China).

Assays for blood glucose and insulin
Blood samples were taken from the venae angularis and left at room temperature for 10 min to allow complete clotting; then they were then centrifuged at 1500 × g for 10 min, followed by the separation of serum from the blood cells. Serum glucose levels were detected by the enzymatic glucose oxidase peroxidase diagnostic kit, and serum insulin was determined by a Sandwich ELISA kit (Nanjing, China).

Morris water maze task
Memory performance was assessed by the MWM test, which consisted of 5-day training (visible and invisible platform training sessions) and a probe trial on day 6. This was carried out as described previously (21). Mice were individually trained in a circular pool (120-cm diameter, 50-cm height) filled to a depth of 30 cm with water maintained at 25°C. The maze was located in a lit room with visual cues. A platform (9-cm diameter) was placed in the center of one quadrant of the pool. The platform’s position was fixed throughout the training sessions; the starting points were pseudo-randomized of each trial, with the animals facing toward the wall. Each mouse was individually trained in both the visible-platform (days 1 – 2) and hidden-platform (days
3 – 5) versions. Visible platform training was performed for baseline differences in vision and motivation; the platform was placed 1-cm below the surface of the water and was indicated by a small flag (5 cm in height). The hidden-platform version evaluates spatial learning and was used to determine the retention of memory to find the platform. During the training, the platform was placed 1 cm below the surface of the water and the flag was removed. The platform was always in the same place. On each day, the animal was subjected to 4 trials with a 1-h interval between trials. Each trial lasted for 90 seconds unless the animal reached the platform first. If an animal failed to find the platform within 90 seconds, the test was ended and the animal was gently navigated to the platform by hand for 30 s. On day 6, the platform was removed and the probe trial started, during which animals had 90 s to search for the platform. The time spent in the target quadrant (i.e., the quadrant where the escape platform was previously located) and the number of target platform crossings was recorded. Data of the escape latency, the time spent in the target quadrant and the number of target platform crossings were collected by the video tracking equipment and processed by a computer equipped with an analysis-management system (Viewer 2 Tracking Software; Ji Liang Instruments, China).

Western blot analysis

Mice hippocampus and cerebral cortex were chopped into small pieces and homogenized in 0.5 ml of RIPA buffer. The dissolved proteins were collected from the supernatant after centrifugation at 12,000 × g for 15 min. Protein concentrations were determined using Coomassie blue-based assay reagent and then Aβ42, APP, BACE1, RAGE, and NF-κB p65 were detected. Protein extracts were separated by a SDS-polyacrylamide gel electrophoresis and then transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk in Tris buffer saline and then incubated at 4°C overnight with the respective primary antibodies for rabbit anti-Aβ42 (1:500), APP (1:500), BACE1 (1:500), RAGE (1:500), NF-κB p65 (1:500), and β-actin (inner control, 1:500). After washing with TBST, the membranes were incubated with a horseradish peroxidase–conjugated secondary antibody (1:2000) for 2 h at room temperature. The antibody-reactive bands were visualized by using the enhanced chemiluminescence detection reagents by a gel imaging system (Tanon Science & Technology Co., Ltd., Shanghai, China).

Statistical analysis

Data shown are expressed as means ± S.E.M. All data were analyzed using one-way ANOVA except for some behavioral data, which were analyzed by two-way ANOVA. Dunnett tests were used for post hoc multiple treatment comparisons. Statistical significance was considered when P < 0.05.

Results

Telmisartan treatment improves memory deficits in STZ-induced diabetic mice

We first evaluated the performance of the diabetic mice in the hippocampus-dependent version of the MWM. Each group of the STZ-induced diabetic mice exhibited similar escape latency in the 2-day visible-platform test, suggesting no differences in vision or basal motivation among all groups [F(5, 239) = 1.327, P = 0.583; Fig. 1A]. We then tested the mice in the 3-day spatial hidden-platform variant; the data showed that diabetic animals without drug treatment showed increases in escape latencies compared to the corresponding controls [F(5, 719) = 6.598, P < 0.05; Fig. 1B], whereas diabetic mice with telmisartan at 0.70 or 0.35 mg/kg displayed significant decreases in escape latencies relative to the untreated diabetic mice (P < 0.05 or P < 0.001, Fig. 1B). In the probe trial conducted 24 h after the last session of acquisition training, a putative measure of spatial learning and memory retention, swim path (Fig. 1C), showed that all the mice exhibited preference for the target quadrant except for the untreated diabetic mice that displayed a significant decrease in the time in the target platform quadrant [F(5, 59) = 22.304, P < 0.05; Fig. 1D] and the number of target platform crossings [F(5, 59) = 19.145, P < 0.05; Fig. 1E] in the target quadrant compared to the control. In contrast, the diabetic mice treated with telmisartan at 0.70 or 0.35 mg/kg showed significant increases in both the time in the target platform quadrant and the number of target platform crossings compared to the untreated diabetic mice (P < 0.01, for the percentage of time and P < 0.05, for the number), indicating that telmisartan (0.70 or 0.35 mg/kg) produced significant improvement on learning and memory deficits in STZ-induced diabetic mice. No significant differences can be observed in behavior tests among the Veh + Veh group and Veh + Tel (0.70 or 0.35 mg/kg) group, suggesting no effects of telmisartan on memory function of normal animals.

Treatment with telmisartan decreases brain Aβ42 in diabetic mice

It has been confirmed that abnormal accumulation of Aβ in the brain is an important cause of memory impairment (9). We examined the brain Aβ42, a more neurotoxic Aβ species, using western blotting. It was shown in Fig. 2 that STZ-induced diabetic mice showed
significant increases in Aβ42 levels of both the hippocampus and cerebral cortex, whereas chronic treatment of telmisartan at 0.70 or 0.35 mg/kg suppressed this increase in the hippocampus [F(3, 15) = 6.115, P < 0.05, Fig. 2: A and B] and cortex [F(3, 15) = 7.983, P < 0.05, Fig. 2: A and B].

Treatment with telmisartan reduces brain APP and BACE1 in the diabetic mice

Aβ is generated by secretase-dependent proteolysis of APP (22). We measured the expression of APP by western blotting. As shown in Fig. 3, the expression of APP was significantly increased in the hippocampus [F(3, 11) = 70.128, P < 0.01] and cortex [F(3, 11) = 182.364, P < 0.01] in STZ-induced diabetic mice. Telmisartan at the dose of 0.70 or 0.35 mg/kg inhibited the APP elevation in both the hippocampus and cortex (P < 0.05, Fig. 3: A and B).

BACE1, also referred to as β-secretase, is a transmembrane aspartic proteinase responsible for cleaving the APP to generate the soluble ectodomain sAPPβ and its C-terminal fragment CTF and thus plays an important role in Aβ production (22). To examine whether BACE1 contributes to the Aβ42 production in STZ-induced diabetic mice, we determined the BACE1 levels by western blotting. As shown in Fig. 3, BACE1 levels significantly increased in the hippocampus [F(3, 11) = 66.352, P < 0.01] and cortex [F(3, 11) = 226.138, P < 0.01] of mice in the STZ + Veh group compared with those of the mice in the Veh + Veh group. Treatment of diabetic mice with telmisartan at 0.70 or 0.35 mg/kg/d caused significant reductions of BACE1 levels in both the hippocampus and the cortex (P < 0.01, Fig. 3: A and C).

Treatment with telmisartan inhibits RAGE and NF-κB p65 in the brains of diabetic mice

Activation of receptor for advanced glycation end...
products (RAGE) may play an important role in Aβ-mediated brain disorder (23). To determine the effect of telmisartan on the brain RAGE of diabetic mice, we detected the expression of RAGE by western blotting. Compared with control mice, the RAGE levels significantly increased in both the hippocampus [F(3, 11) = 167.227, *P < 0.01] and cortex [F(3, 11) = 44.653, *P < 0.01] of diabetic mice. Treatment of diabetic mice with telmisartan at 0.70 or 0.35 mg/kg showed marked decreases of RAGE expression in both the hippocampus and cortex (*P < 0.05, Fig. 4: A and B).

Because activation of RAGE triggers NF-κB signaling that regulates some gene expression such as APP, BACE1, etc. (24), we used western blotting to examine NF-κB p65 levels in the brain. The present data showed that NF-κB p65 levels significantly increased in the hippocampus [F(3, 15) = 9.337, *P < 0.01] and cortex [F(3, 15) = 6.135, *P < 0.01] in STZ-induced diabetic mice compared to control mice. Chronic treatment with telmisartan (0.70 or 0.35 mg/kg) significantly decreased NF-κB p65 levels in both the hippocampus and cortex (*P < 0.01, Fig. 4: A and C) compared to those of diabetic mice without drug treatment.

Fig. 2. Telmisartan treatment reduces brain Aβ42 accumulation in STZ-induced diabetic mice. Representative immunoblots of Aβ42 and β-actin (inner control) were obtained by western blotting using the appropriate antibodies (A), and quantification of Aβ42 is expressed as a proportion (in percentage) relative to the control (B). Values are expressed as means ± S.E.M. (n = 3 – 5). *P < 0.05, **P < 0.01 vs. STZ plus Veh for hippocampus; †P < 0.05, ‡P < 0.01 vs. STZ plus Veh for cortex.

No effects of telmisartan on body weight, blood pressure, blood glucose, and insulin in the diabetic mice

After five-week treatment of telmisartan, no significant differences were observed in body weight among the diabetic mice of each group. Telmisartan at 0.70 or 0.35 mg/kg didn’t change blood glucose, insulin and blood pressure in the diabetic mice (P > 0.05, Table 1).
The frequently used model of type 1 diabetes, STZ-induced diabetic animals, is characterized by chronic hyperglycemia associated with impaired hippocampus-dependent learning and memory as well as defective synaptic plasticity in the hippocampus (25). The mechanisms linking diabetes to cognitive dysfunction are still unclear. Mounting evidence showed abnormal accumulation of Aβ in the brain is involved in diabetes-associated cognitive dysfunction. It is well known that Aβ is a cleavage product derived from APP, and on sequential cleavage by aspartyl proteases β-secretase and γ-secretase. APP is processed to generate various peptide species, including the more toxic form Aβ_{42} that is prone to oligomerization, leading to neurotoxicity (26). In the present study, STZ-induced diabetic mice characterized by hyperglycemia and hypoinsulinemia demonstrated marked deficits in memory function, which was coupled with significant increases in Aβ_{42}, a key Aβ species for memory damage, in the hippocampus or cortex. Chronic treatment with telmisartan significantly ameliorated memory deficits and produced significant decreases in Aβ_{42} levels in the hippocampus or cortex. Telmisartan treatment also reduced APP, BACE1, RAGE, and NF-κB_{p65} in the brain, but did not attenuate hyperglycemia or hypoinsulinemia in STZ-induced diabetic mice.

Hyperglycemia, a consequence of diabetes, enhances the formation of advanced glycation end products (AGEs), senescent protein derivatives that result from the auto-oxidation of glucose and fructose (27). Binding of AGEs to the receptor for AGEs (RAGE) activates intracellular signaling processes, such as the NF-κB pathway, thus regulating expression of some genes. The formation and accumulation of AGEs have been known to progress at an accelerated rate in diabetes, which is a pivotal player in diabetic complications (28, 29). It has been reported both APP and BACE1 promoter contain a binding site for NF-κB_{p65} (24, 30) and expression of both APP and BACE1 was markedly increased in diabetic animals (31 – 33). Thus, activation of the AGEs/RAGE axis, as a result of hyperglycemia, drives the upregulation of the APP and BACE1 for

### Table 1. No effects of telmisartan on body weight, blood glucose, insulin, and blood pressure in STZ-induced diabetic mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Blood glucose (mM)</th>
<th>Serum insulin (mIU/L)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh + Veh</td>
<td>28.67 ± 1.25</td>
<td>6.71 ± 0.75</td>
<td>14.21 ± 0.600</td>
<td>84.39 ± 3.13</td>
</tr>
<tr>
<td>STZ + Veh</td>
<td>21.55 ± 1.17**</td>
<td>18.21 ± 1.23**</td>
<td>5.36 ± 0.19**</td>
<td>83.74 ± 2.78</td>
</tr>
<tr>
<td>STZ + Tel (0.70 mg/kg)</td>
<td>22.10 ± 1.52**</td>
<td>17.43 ± 1.85**</td>
<td>5.88 ± 0.25**</td>
<td>84.15 ± 2.32</td>
</tr>
<tr>
<td>STZ + Tel (0.35 mg/kg)</td>
<td>22.32 ± 1.33**</td>
<td>17.88 ± 1.67**</td>
<td>5.75 ± 0.27**</td>
<td>83.09 ± 2.02</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. (n = 7 – 8). **P < 0.01 vs. Veh + Veh.
Aβ production via NF-κB activation and results in Aβ accumulation in diabetic animals. In addition, the increased AGEs or Aβ continued binding RAGE, which, in turn, caused NF-κB activation, thereby triggering a positive feedback loop in which RAGE expression is upregulated and thus enhances the binding capacity of the AGEs and Aβ ligands (34). As expected, activation of the NF-κB pathway, which is characterized by NF-κB p65 elevation, and its mediated-upregulation of APP, BACE1, and RAGE were observed in STZ-induced diabetic mice.

The renin–angiotensin system (RAS) contributes to the development and progression of diabetic encephalopathy as well (35). Importantly, there exists the cross-talk between the RAS and the AGE–RAGE system (36). It was demonstrated that AT1R signaling contributes to diabetes-induced neuroinflammation such as increases of angiotensin II and intercellular adhesion molecule-1 (ICAM-1), leukocyte adhesion, etc. (35, 37). Angiotensin II functions as a proinflammatory factor to induce activation of the NF-κB pathway and upregulation of RAGE (36, 38). Angiotensin II is a final product of the RAS produced from angiotensinogen through enzymatic cascade reactions, and the RAS components required for the generation of angiotensin II are reported to exist in the brain (39, 40). Angiotensin II mediates several key events of the inflammatory processes. Binding of angiotensin II to its receptors (in particular AT1R) mediates proinflammatory molecule production and free radical generation that contribute to tissue damage, and blocking angiotensin II signaling protects against neurodegenerative processes (41). Telmisartan is an ARB with a high degree of lipophilicity and is able to cross the blood–brain barrier (BBB). Recently it has been reported that telmisartan shows cerebroprotective effects, improves cognitive decline in AD, and provides neuroprotection (20, 42, 43); and these actions of telmisartan are attributed to its potential anti-oxidative (13), anti-inflammatory (12), and anti-Aβ (20) effects. In the study, telmisartan at either 0.7 or 0.35 mg/kg body weight did not produce a hypotensive effect confirmed by blood pressure recording. A non-hypotensive dose of telmisartan preferred to block binding of Angiotensin II to cerebral AT1R because of the higher distribution of this receptor in the brain, which results in decrease of NF-κB signaling. Additionally, telmisartan is a unique ARB with a partial peroxisome proliferator–activated receptor-gamma (PPARγ) agonistic property (44), and PPARγ can block NF-κB-dependent gene expression through co-repressor interference (45). Thus, it is much reasonable that decreases in the expression of APP, BACE1, or RAGE resulted from inhibition of NF-κB signaling in the brains of diabetic mice treated with telmisartan.

In conclusion, we have demonstrated that chronic administration of telmisartan improves learning performance in STZ-induced diabetic mice. This CNS effect results from alleviation of amyloidosis in the brain rather than attenuation of hyperglycemia and hypoinsulinemia in the periphery. Our study has important implications for patients with type 1 diabetes, suggesting a potential role for telmisartan as an adjuvant therapy for the prevention and treatment of diabetes-associated memory deficits.

Acknowledgments

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Conflicts of Interest

None of the authors have any financial interests to disclose.

References


