Peroxisome Proliferator-Activated Receptor-γ Activation With Angiotensin II Type 1 Receptor Blockade Is Pivotal for the Prevention of Blood-Brain Barrier Impairment and Cognitive Decline in Type 2 Diabetic Mice

Li-Juan Min, Masaki Mogi, Masachika Shudou, Fei Jing, Kana Tsukuda, Kousei Ohshima, Jun Iwanami, Masatsugu Horiuchi

Abstract—We reported previously that an angiotensin II type 1 receptor blocker, telmisartan, improved cognitive decline with peroxisome proliferator-activated receptor-γ activation; however, the detailed mechanisms are unclear. Enhanced blood-brain barrier (BBB) permeability with alteration of tight junctions is suggested to be related to diabetes mellitus. Therefore, we examined the possibility that telmisartan could attenuate BBB impairment with peroxisome proliferator-activated receptor-γ activation to improve diabetes mellitus-induced cognitive decline. Type 2 diabetic mice KKAy exhibited impairment of cognitive function, and telmisartan treatment attenuated this. Cotreatment with GW9662, a peroxisome proliferator-activated receptor-γ antagonist, interfered with these protective effects of telmisartan against cognitive function. BBB permeability was increased in both the cortex and hippocampus in KKAy mice. Administration of telmisartan attenuated this increased BBB permeability. Coadministration of GW9662 reduced this effect of telmisartan. Significant decreases in expression of tight junction proteins and increases in matrix metalloproteinase expression, oxidative stress, and proinflammatory cytokine production were observed in the brain, and treatment with telmisartan restored these changes. Swollen astroglial end-feet in BBB were observed in KKAy mice, and this change in BBB ultrastructure was decreased in telmisartan. These effects of telmisartan were weakened by cotreatment with GW9662. In contrast, administration of another angiotensin II type 1 receptor blocker, losartan, was less effective compared with telmisartan in terms of preventing BBB permeability and astroglial end-foot swelling, and coadministration of GW9662 did not affect the effects of losartan. These findings are consistent with the possibility that, in type 2 diabetic mice, angiotensin II type 1 receptor blockade with peroxisome proliferator-activated receptor-γ activation by telmisartan may help with protection against cognitive decline by preserving the integrity of the BBB.

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Key Words: AT1 receptor blockade ■ blood-brain barrier ■ cognitive decline ■ diabetes ■ PPAR-γ activation ■ swollen astrocytic end-feet
BBB permeability, implicating the involvement of BBB impairment in the progression of T2DM and subsequent cognitive decline. Indeed, recent animal studies using the streptozotocin-induced diabetes mellitus model and clinical studies have indicated that diabetes mellitus increases BBB permeability with downregulation of TJ protein expression. These reports suggest that BBB impairment might play a causal role in T2DM-induced cognitive decline.

Angiotensin II via its type 1 (AT1) receptor stimulation may contribute to the pathogenesis of CNS disorders and cognitive decline by promoting hypertension, vascular inflammation, oxidative stress, and neuronal damage. AT1 receptor blockers (ARBs) could reduce the incidence and progression of stroke, Alzheimer disease, and dementia.

In a T2DM mouse model, KKAy, we reported that an ARB prevented cognitive decline with an increase in cerebral blood structural and functional changes in T2DM, leading to the recently that telmisartan exerted a preventive effect on functional ARBs. Some ARBs, such as telmisartan and losartan (1 mg/kg per day by oral injection) and/or terted telmisartan or losartan (1 mg/kg per day by oral injection) and/or

Animals and Treatment
Adult male KKAy mice and C57BL/6J mice (CLEA, Tokyo, Japan) were used in this study. Telmisartan was provided by Boehringer Ingelheim (Ingelheim, Germany). Losartan was purchased from LKT Laboratories (St Paul, MN), and GW9662 was purchased from Sigma-Aldrich (St Louis, MO). Mice were kept in a room in which lighting was controlled (12 hours on, 12 hours off), and the temperature was maintained at 25°C. They were given a standard diet (MF, Oriental Yeast, Tokyo, Japan) and water ad libitum. KKAy mice were administered telmisartan or losartan (1 mg/kg per day by oral injection) and/or

| Table. BW, SBP, and Glucose Level in Each Group |
|------------------|------------------|------------------|------------------|
| Characteristics  | C57BL/6J         | Control          | Tel              | Tel+GW           | GW               |
| BW, g            | 28.4±1.2         | 46.6±0.9         | 47.1±0.8         | 45.8±1.2         | 46.2±1.4         |
| SBP, mm Hg       | 96.0±1.2         | 102.4±2.0        | 100.5±1.8        | 100.8±1.6        | 101.6±1.9        |
| Glucose (nonfasting), mg/dL | 157.8±15.1 | 416.4±38.2*     | 408.2±32.7*     | 410.8±37.5*     | 404.1±45.3*     |
| Glucose (fasting), mg/dL  | 77.2±6.4     | 120.3±10.7*     | 115.0±5.6*      | 123.3±11.8*     | 118.5±7.3*      |

Body weight (BW), systolic blood pressure (SBP), and plasma glucose level in nonfasting state and 16 h of fasting state were measured 7 wk after each group treatment. Tel indicates telmisartan (1 mg/kg per d); GW, GW9662 (0.35 mg/kg per d); n=6 to 8 for each group.

*P<0.01 vs C57BL/6J at nonfasting and fasting state, respectively.

Morris Water Maze Test
Cognitive function was evaluated by the Morris water maze test, as described previously. Each treated mouse was trained 5 times a day at 20-minute intervals. The test was performed blindly, every day for 5 days. Swimming was video tracked (AnyMaze, Wood Dale, IL), and latency, path length, swim speed, and cumulative distance from the platform were recorded. Mean swim latency for all of the trials on each day in each group was calculated. After a probe trial, the mean time spent in the correct quadrant containing the platform and the mean number of times that mice crossed the former platform position during 60 seconds were determined.

Evaluation of BBB Permeability
BBB permeability was evaluated by measuring extravasation of Evans Blue (EB) dye, which can bind to serum albumin after intravenous injection and, therefore, has been used as a tracer for serum albumin. Briefly, EB dye (2% in saline, 4 mL/kg) was injected intravenously via the tail and allowed to circulate for 3 hours. Mice were perfused transcardially with ice-cold PBS to remove the intravascular dye. After decapitation, the brain was removed and divided into 4 regions, left cerebral cortex, right cerebral cortex, left hippocampus, and right hippocampus. Then each region was weighed for quantitative measurement of EB-albumin extravasation. Samples were homogenized in 2.5 mL of PBS and mixed with 2.5 mL of 60% trichloroacetic acid. Then samples were cooled for 30 minutes followed by centrifugation (4°C, 1000 g, 30 minutes) to precipitate the protein. The supernatant was subjected to measurement of the absorbance of EB at 610 nm using a spectrophotometer. EB dye was expressed as micrograms per milligram of brain tissue against a standard curve. For macroscopic evaluation, after decapsulation and perfusion with ice-cold PBS, the brain was rapidly removed and coronally sectioned at ~1-mm thickness and photographed to observe EB dye extravasation.

We also measured extravasation of the fluorescent dye albumin-Allexa Fluor-488 (Invitrogen) and IgG-DyLight549 (Jackson ImmunoResearch Laboratories, Inc) to evaluate BBB permeability. Albumin-Allexa Fluor-488 (25 mg/mL in saline) and IgG-DyLight549 (1.5 mg/mL in saline) were mixed and injected intravenously via the tail and allowed to

**Methods**
This study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. All of the animal studies were reviewed and approved by the Ehime University Animal Studies Committee.

**Animals and Treatment**
Adult male KKAy mice and C57BL/6J mice (CLEA, Tokyo, Japan) were used in this study. Telmisartan was provided by Boehringer Ingelheim (Ingelheim, Germany). Losartan was purchased from LKT Laboratories (St Paul, MN), and GW9662 was purchased from Sigma-Aldrich (St Louis, MO). Mice were kept in a room in which lighting was controlled (12 hours on, 12 hours off), and the temperature was maintained at 25°C. They were given a standard diet (MF, Oriental Yeast, Tokyo, Japan) and water ad libitum. KKAy mice were administered telmisartan or losartan (1 mg/kg per day by oral injection) and/or

GW9662 (0.35 mg/kg per day in drinking water), a PPAR-γ antagonist, for 7 weeks from 8 weeks of age. Telmisartan was dissolved in sterilized water with carboxymethylcellulose sodium salt at a concentration of 1 mg/kg per day, and losartan was dissolved in sterilized water at a concentration of 1 mg/kg per day. Then, 0.1 mL of solution was administered to each mouse. Vehicle solution (0.1 mL) was also administered to control mice. We calculated the mean amount of daily drinking water of mice, which was necessary to give GW9662 at a dose of 0.35 mg/kg per day in each group. We did not find significant differences in drinking water volume among each group. We observed that the dose of telmisartan or GW9662 used did not show significant effects on body weight, systolic blood pressure, and plasma glucose level in both a nonfasting and fasting state (Table). The effect of telmisartan or GW9662 on oral glucose tolerance test was shown in Figure S1 (available in the online-only Data Supplement).
circulate for 16 hours. After perfusion with ice-cold PBS, the brain was removed and coronally sectioned at ~1-mm thickness and photographed to determine the distribution of injected dyes at X40 magnification. The intensity of fluorescent IgG-DyLight549 outside vessels was quantified using computer-imaging software (Densitograph, ATTO Corporation, Tokyo, Japan).

**Western Blot Analysis**
Total protein was prepared from the cerebral cortex and hippocampus of the mouse brain. The proteins were subjected to SDS-PAGE and immunoblotted with antizonula occluden (ZO)-1 antibody, antioccludin antibody, antialcinudin 3 antibody, antialcinudin 5 antibody (Invitrogen), antivascular endothelial (VE)-cadherin antibody (R&D Systems, Inc), antimatrix metalloproteinase (MMP) 2 antibody, anti–MMP-9 antibody, or anti–β-actin antibody (Sigma-Aldrich, Inc, St Louis, MO). Visualization of proteins and densitometric analysis were performed as described previously.

**Transmission Electron Microscopic Examination**
Anesthetized mice were perfused through the left ventricle with 15 mL of PBS followed by 60 mL of 4.0% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L of cacodilic acid buffer (pH 7.3). The fixed brain was dehydrated through an ethanol series, embedded in epoxy resin, and cut into ultrathin sections. The sections were mounted on copper grids, stained in uranyl acetate and citric acid lead, and then observed under a transmission electron microscope (JEM1230; JEOL Ltd, Tokyo, Japan) in the Integrated Center for Science of Ehime University.

**Monocyte Chemoattractant Protein 1, Tumor Necrosis Factor-α, and Interleukin 6 Protein Assay**
Total protein was prepared from mouse brain. The levels of monocyte chemoattractant protein 1, tumor necrosis factor-α, and interleukin 6 in these samples were measured using commercially available ELISA kits (interleukin 6, from Bender MedSystems, Inc, Burlingame, CA; monocyte chemoattractant protein 1, from R&D Systems, Minneapolis, MN; tumor necrosis factor-α, from Shibayagi, Inc, Gunma, Japan) following the manufacturers’ instruction. The concentration of each marker was calculated by comparison with standard curves. Volumes indicate the concentrations of cytokines in milligrams per milliliter and are normalized to total protein and expressed as picograms per milligram of protein.

**Detection of Superoxide Anion in Brain**
Superoxide anion production was detected as described previously. Frozen sections of the brain were stained with dihydroethidium (10 μmol/L) in PBS for 30 minutes at 37°C in a humidified chamber protected from light. For detection of ethidium, samples were examined with a Leica DMi6000B (Leica Microsystems, Wetzlar, Germany) equipped with a computer-based imaging system (FW4000, Leica). The intensity of the fluorescence was analyzed and quantified using computer-imaging software (Densitograph, ATTO Corporation, Tokyo, Japan).

**Materials**
Reagents not mentioned above were purchased from Sigma-Aldrich.

**Statistical Analysis**
All of the values are expressed as mean±SEM in the text, and figure data were evaluated by ANOVA followed by post hoc analysis for multiple comparisons. Differences with P<0.05 were considered significant.

**Results**

**Effect of AT1 Receptor Blockade With PPAR-γ Activation on Cognitive Function in KKAγ Mice**
We examined the effects of telmisartan on cognitive function in KKAγ mice using the Morris water maze test. KKAγ mice at 15 weeks of age showed impairment of acquisition of spatial memory determined by escape latency in the Morris water maze test compared with age-matched C57BL/6J mice (Figure 1A and 1B). In the probe trial, the time spent in the correct quadrant and the frequency of crossing to the former platform place at 5 days of Morris water maze test were both decreased in KKAγ mice (Figure 1C and 1D), suggesting that KKAγ mice cannot develop enhanced spatial memory and undergo cognitive decline. Treatment of KKAγ mice with telmisartan markedly improved these parameters time dependently with exercise along with the Morris water maze test, thereby attenuating the cognitive decline. Cotreatment with GW9662 significantly prevented this improvement of cognitive decline by telmisartan.

**Effect of AT1 Receptor Blockade With PPAR-γ Activation on BBB in KKAγ Mice**
To investigate whether telmisartan affected BBB via a partial PPAR-γ agonistic action in KKAγ mice with cognitive decline, we studied BBB permeability and its structure in comparison with the effects of another ARB, losartan, which has less PPAR-γ agonistic activity. Figure 2A shows that accumulation of EB dye in both the cerebral cortex and hippocampus, especially in the striatum, was increased in KKAγ mice compared with that in C57BL/6J mice, indicating that T2DM induced BBB permeability. Administration of telmisartan attenuated this increase in BBB permeability, and coadministration of GW9662 significantly reduced this telmisartan-mediated improvement of BBB permeability, whereas GW9662 alone did not influence accumulation of EB (Figure 2A and 2B). In contrast, treatment with losartan was less effective, and coadministration of GW9662 did not affect the effect of losartan (Figure 2B). Moreover, we observed significant extravasation of the fluorescent dye albumin-Alexa Fluoro-488 (green) or IgG-DyLight549 (red) around small blood vessels in the brain in KKAγ mice. Treatment with telmisartan decreased the extravasation of albumin-Alexa Fluoro-488 or IgG-DyLight549, and coadministration of GW9662 attenuated this effect of telmisartan (Figure 2B).

We also examined ultrastructural changes of the BBB in the brain using transmission electron microscopy. Astrocyte endfeet were swollen to various degrees in the small vessels of the EB-accumulated regions of the brain in KKAγ (Figure 3a and 3b). Moreover, detachment of the end feet plasma membrane from the basolateral membrane was also observed in KKAγ mice (Figure 3a and 3b). Interestingly, such distinctive changes were not obvious in KKAγ mice treated with losartan (Figure 3c and 3d). Losartan treatment was less effective in preventing ultrastructural changes of the BBB (Figure 3i).

Coadministration of GW9662 partially prevented the telmisartan-mediated improvement of swollen astrocytic endfeet and astrocytic detachment (Figure 3e and 3f) but did not affect the effect of losartan (Figure 4J). Treatment with GW9662 alone had no significant effects on these ultrastructural changes of the BBB in KKAγ mice (Figure 3g and 3h). TJ proteins are closely associated with BBB function, so we next examined the effects of telmisartan on the expression of TJ proteins in the brain. Significant decreases in expression of ZO-1, occludin, claudin 3, and claudin 5...
were observed in both the cerebral cortex and hippocampus of KKAy mice compared with C57BL/6J mice. Treatment with telmisartan inhibited these decreases in TJ protein expression. Cotreatment with GW9662 prevented these inhibitory effects of telmisartan, whereas GW9662 alone did not change TJ protein expression (Figure 4).

Because the assembly of AJ is required for the correct organization of TJ to perform the barrier function of brain endothelial cells, we also analyzed the expression of VE-cadherin, which is an endothelium-specific member of the cadherin family of AJ proteins, and obtained similar results to TJ protein expression (Figure 4).

Effect of Telmisartan on MMPs, Oxidative Stress, and Inflammation
To further address the possible involvement of MMPs, oxidative stress, and inflammation in telmisartan-ameliorated TJ and AJ protein expression, we also assessed MMP-2 and MMP-9 expression. Both the cerebral cortex and hippocampus of KKAy mice showed increased expression of both MMP-2 and MMP-9 compared with those of C57BL/6J mice. Administration of telmisartan significantly reduced these increases in MMPs expression, whereas coadministration of GW9662 inhibited these effects of telmisartan (Figure 4). Moreover, the brain in KKAy mice exhibited enhanced...
Figure 2 (Continued).
expression of monocyte chemotactant protein 1, tumor necrosis factor-α, and interleukin 6 and increased superoxide production. Treatment with telmisartan inhibited these increases of proinflammatory cytokines and superoxide production, and cotreatment with GW9662 attenuated these effects of telmisartan (Figure 5).

**Discussion**

Impairment of the BBB is a critical event in the development and progression of several diseases that affect the CNS. We demonstrated here that increased BBB permeability with downregulation of TJ and AJ proteins was involved in T2DM-induced cognitive impairment. TJs present between cerebral endothelial cells perform barrier functions of the BBB and consist of many proteins, such as claudin 3, claudin 5, occludin, and ZO-1. On the other hand, AJs are required for the correct organization of TJs and are largely composed of VE-cadherin in endothelial cells. Therefore, alteration of the interaction between these TJ proteins and VE-cadherin plays an essential role in modulating BBB function. Our results are consistent with these ideas and previous observations, suggesting that reduced ZO-1 and occludin expression, for example, might contribute to enhanced BBB permeability in diabetes mellitus. However, further alterations of BBB ultrastructure in diabetic patients or animal models are a matter of debate. Astrocytic end-foot ensheathe the vessel wall in the brain and appear to be critical for induction and maintenance of the TJ barrier. We assessed ultrastructural changes of the BBB by transmission electron microscopic examination, revealing swollen astrocytic end-foot in diabetic mice with cognitive decline. Our findings strongly support the notion that BBB structural and functional changes are involved in diabetes mellitus, leading to cognitive decline.

Recently, the therapeutic effectiveness of ARBs with selective PPAR-γ modulation has been expected to prevent stroke and metabolic syndrome with 2 distinct beneficial effects. Telmisartan is reported to prevent metabolic syndrome through a partial PPAR-γ agonistic effect, as well as an AT₁ receptor-blocking action. Recently, we have demonstrated that telmisartan also protected against cognitive decline in a mouse model of Alzheimer disease through AT₁ receptor blockade and PPAR-γ activation. Here, we further explored the mechanisms involved in the telmisartan-

**Figure 2 (Continued).** Effect of angiotensin II type 1 (AT₁) receptor blockade with peroxisome proliferator-activated receptor (PPAR)-γ activation on blood-brain barrier (BBB) function in KKAy mice. A and B, BBB permeability determined by measuring extravasation of Evans Blue (EB) dye. After injection of EB (2% in saline, 4 mL/kg), the left and right cerebral cortex and left and right hippocampus of mice were subjected to quantification of EB-albumin extravasation by trichloroacetic acid precipitation method and absorbance reading. A, Representative photographs of EB accumulation in coronal sections of mouse brain. B, Quantification of accumulation of EB dye in each region of mouse brain. n=6 to 8 for each group. aP<0.05, bP<0.05, cP<0.05, dP<0.05, eP<0.05, fP<0.05, gP<0.05, hP<0.05 vs KKAy mice. KKAy+Tel and KKAy+Tel+GW.

**Figure 3.** Effect of angiotensin II type 1 (AT₁) receptor blockade with peroxisome proliferator-activated receptor (PPAR)-γ activation on ultrastructural changes of blood-brain barrier (BBB) shown by transmission electron microscopy in KKAy mice (a and b), KKAy+Tel (c and d), KKAy+Tel+GW (e and f), KKAy+GW (g and h), KKAy+Los (i), and KKAy+Los+GW (j). a1 through h1 are higher magnification images of the squares shown in each figure from a to h. Triangles (α) indicate astrocytic end-feet. White arrows indicate cell detachment. Black arrow indicates broken basal membrane. Black bars indicate 2 μm. Telmisartan (Tel) or losartan (Los) was given at 1 mg/kg per day by oral injection, and GW9662 (GW) was given at 0.35 mg/kg per day in drinking water for 7 weeks. Similar results were obtained in 6 to 8 mice in each group.

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6 Hypertension May 2012
Figure 4. Effect of angiotensin II type 1 (AT₁) receptor blockade with peroxisome proliferator-activated receptor (PPAR)-γ activation on tight junction (TJ) and adherens junction (AJ) protein and matrix metalloproteinase (MMP) expression in KKAy mice. Total protein extracted from the hippocampus and cortex of mice was subjected to Western blot analysis. Representative photographs from different experiments are shown in A. Densitometric measurements of the bands are shown in B. Tel indicates telmisartan (1 mg/kg per day by oral injection); GW, GW9662 (0.35 mg/kg per day in drinking water) for 7 weeks. n=7 for each group. *P<0.01 vs C57BL/6J; †P<0.01 vs KKAy; #P<0.05 vs KKAy+Tel.
mediated improvement of cognitive function. The present findings demonstrated that telmisartan with a partial PPAR-γ agonistic effect may have a protective effect even on ultrastructural changes in BBB impairment, because telmisartan ameliorated BBB function; restored the expression of ZO-1, occludin, claudin 3, claudin 5 and VE-cadherin; and decreased astrocytic end-feet swelling, and these effects of telmisartan were inhibited by GW9662, a PPAR-γ antagonist.

There are some articles reporting that AT1 receptor blockade protected BBB function. Fleegal-DeMotta et al.24 reported that telmisartan inhibited angiotensin II–induced BBB permeability in endothelial cells, contributing to the prevention of hypertensive encephalopathy. Furthermore, Pelisch et al.25 suggested that the ARB olmesartan had neuroprotective effects in cognitive disorders by preventing BBB permeability. However, in those studies, the inhibition of ultrastructural changes of the BBB by ARBs was not examined, and whether PPAR-γ activation was involved in the preventive effect of ARBs on BBB permeability was not clarified. Our results demonstrated that AT1 receptor blockade with telmisartan could prevent T2DM-associated cognitive decline at least via upregulation of TJ protein expression and amelioration of astrocytic end-feet swelling in concert with PPAR-γ activation. Telmisartan showed more pronounced induction of PPAR-γ activation at lower, pharmacologically relevant concentrations compared with the other ARBs, and losartan enhanced PPAR-γ activation only at high concentrations.16 Therefore, we used another ARB, losartan, as the control to clarify whether PPAR-γ activation is involved in the protective effects of telmisartan against BBB permeability and

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**Figure 5.** Production of proinflammatory cytokines (A) and superoxide anion (B) in the brain. Total protein was subjected to analysis of the protein level of each proinflammatory cytokine using ELISA. Data are normalized to total protein and expressed as picograms per milligram of protein (A). Superoxide anion production was determined by dihydroethidium staining (B). Top lanes show representative photos, and bottom lanes show fluorescence intensity in arbitrary units. Tel indicates telmisartan (1 mg/kg per day by oral injection); GW, GW9662 (0.35 mg/kg per day in drinking water) for 7 weeks. n=7 for each group. *P<0.01 vs C57BL/6J; †P<0.01 vs KKAy; #P<0.05 vs KKAy+Tel.
cognitive decline. In comparison with telmisartan, losartan had less inhibitory effects on diabetes mellitus–associated BBB functional and ultrastructural changes, and cotreatment with GW9662 did not affect these effects of losartan. These findings demonstrated that AT$_1$ receptor blockade by telmisartan prevents T2DM-associated BBB dysfunction and cognitive decline at least in part through PPAR-$\gamma$ activation, supporting the notion that some ARBs (so-called metabosartans)$^{26,27}$ with a partial PPAR-$\gamma$ agonistic effect could have pleiotropic effects beyond the AT$_1$ receptor blockade of ordinary ARBs.

MMPs are zinc-dependent proteinases that can degrade numerous structural components of the extracellular matrix and non-extracellular matrix proteins.$^{28}$ Expression and activity of the gelatinases MMP-2 and MMP-9 were enhanced by hyperglycemia in vitro$^{29}$ and elevated in patients with type 1 and type 2 diabetes mellitus.$^{30,31}$ Moreover, recent data suggested roles of MMPs in regulating TJ proteins. In streptozotocin-treated rats, Navaratna et al$^{32}$ demonstrated that MMP-2 and MMP-9 in telmisartan-mediated restoration of TJ protein expressions. In contrast to TJ proteins, both MMP-2 and MMP-9 expressions were increased in KKA$^\text{Y}$ mice. Administration of telmisartan significantly reduced the increased expression of these MMPs, whereas coadministration of GW9662 inhibited these effects of telmisartan. Our results indicated 1 possible mechanism involved in telmisartan-mediated regulation of TJ protein expression involving PPAR-$\gamma$ activation, which is associated with MMP-2- and MMP-9–mediated proteolytic processes.

It is well known that inflammation plays critical roles in the pathogenesis of vascular injury and BBB dysfunction.$^8$ A recent clinical study showed that elevated circulating levels of inflammatory markers were associated with poorer cognitive function in a population with T2DM.$^{33}$ It has been reported that AT$_1$ receptor blockade decreases nuclear factor-$\kappa$B activation with PPAR-$\gamma$ activation in the vasculature.$^{34}$ Moreover, PPAR-$\gamma$ stimulation is known to suppress AT$_1$ receptor expression in vascular smooth muscle cells and plays a regulatory role in expression of genes involved in inflammatory response.$^{35,36}$ We observed that the brain in KKA$^\text{Y}$ mice showed enhanced expression of proinflammatory cytokines and increased superoxide production. Telmisartan inhibited expression of these proinflammatory cytokines and superoxide production in KKA$^\text{Y}$ mice, and these effects of telmisartan were attenuated by GW9662. These observations suggest that antioxidative and anti-inflammatory effects of telmisartan involving PPAR-$\gamma$ stimulation could contribute to improvement of T2DM-induced BBB impairment and cognitive function. Consistent with these ideas, the anti-inflammatory and antioxidative effects of telmisartan with PPAR-$\gamma$ activation are reported to be closely associated with its protective effects against cognitive impairment and white matter damage after chronic cerebral hypoperfusion.$^{37}$ Moreover, it has been demonstrated that oxidative stress can activate MMP-2 and MMP-9 via tyrosine kinase–dependent mechanisms, leading to BBB breakdown.$^{38}$ Proinflammatory cytokines are also suggested to induce the release of MMP-9 from brain pericytes in the damaged BBB.$^{39}$ Therefore, in T2DM, the accumulation of oxidative stress and inflammation response induce and activate MMPs, which further downregulate TJ proteins and promote swollen astrocytic end-feet, and ultimately lead to BBB dysfunction and subsequent cognitive decline. Telmisartan with PPAR-$\gamma$ activation can protect against this BBB impairment and cognitive decline via prevention of enhanced MMP activity mediated by its antioxidative and anti-inflammatory effects and further upregulation of TJ protein expression and amelioration of astrocytic end-feet swelling. However, further studies are necessary to clarify the precise mechanistic relationships between any anti-inflammatory and antioxidant effects of telmisartan, MMPs, TJ proteins, and BBB and cognitive function in the setting of T2DM.

In the present study, we did not observe any blood pressure changes in the different experimental groups. This suggests that AT$_1$ receptor blockade with PPAR-$\gamma$ activation can attenuate cognitive decline and preserve BBB integrity independent of effects on blood pressure. Moreover, mechanistic studies suggest that vascular disease and alterations in glucose, insulin, and amyloid metabolism underlie the pathophysiology of cognitive impairment in diabetes mellitus.$^{40}$ Hyperglycemia in diabetes mellitus could result in oxidative stress accumulation and inflammatory response,$^7$ and these hyperglycemia-related events have been demonstrated to cause BBB structural changes and increase BBB permeability.$^8$ We observed that KKA$^\text{Y}$ mice with cognitive decline exhibited higher plasma glucose concentration in the fed state and fasting state compared with wild-type mice. However, the plasma glucose level under these conditions was not significantly changed in KKA$^\text{Y}$ mice treated with telmisartan with or without GW9662 (Table). We also observed that telmisartan treatment attenuated the glucose level in KKA$^\text{Y}$ mice in an oral glucose tolerance test (30 and 60 minutes after glucose administration), and cotreatment with GW9662 inhibited this effect of telmisartan (Figure S1). Therefore, it is also possible that the improvement of insulin resistance and glucose intolerance by telmisartan could also contribute to its preventive effects on BBB dysfunction and cognitive decline in addition to its direct protective effects on BBB in T2DM.

In conclusion, the present study demonstrated that AT$_1$ receptor blockade and partial PPAR-$\gamma$ activation by telmisartan upregulated TJ protein expression, attenuated swelling of astrocytic end-feet, and limited BBB permeability via downregulation of MMPs mediated by its antioxidative and anti-inflammatory effects. These effects might contribute to the observed ability of telmisartan to protect against cognitive decline in T2DM.

Perspectives
These findings contribute to further understanding of the pathophysiologic effects of BBB permeability in diabetes mellitus and suggest a potential therapeutic approach to protect against diabetes mellitus–induced cognitive decline. Additional mechanistic and pharmacological studies of the beneficial effects of AT$_1$ receptor blockade and PPAR-$\gamma$ activation on BBB permeability and cognitive function in diabetes mellitus could be helpful for the development of more therapeutically effective
ARBs in the future. Such studies could ultimately lead to new opportunities for the prevention and treatment of cognitive decline in patients with T2DM.

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Disclosures
None.

References
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PPAR-γ Activation with AT₁ Receptor Blockade is Pivotal for Prevention of Blood-Brain Barrier Impairment and Cognitive Decline in Type 2 Diabetic Mice

Short title: ARB with PPAR-gamma Agonist Prevents BBB Disruption

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**Figure S1**

Effect of telmisartan on oral glucose tolerance test (OGTT). OGTT was performed after fasting for 16 h. Glucose solution (2 g/kg) was administered orally, and a small amount of blood was obtained from the tail vein without anesthesia at 0, 30, 60 and 120 min. Data are expressed as mean ± SEM. *P<0.05 vs. C57BL/6J; †P<0.05 vs. KKA\(^y\); #P<0.05 vs. KKA\(^y\)+Tel.