Nonhypotensive Dose of Telmisartan Attenuates Cognitive Impairment Partially Due to Peroxisome Proliferator-Activated Receptor-γ Activation in Mice With Chronic Cerebral Hypoperfusion

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Background and Purpose—The effect of telmisartan, an angiotensin II Type 1 receptor blocker with peroxisome proliferator-activated receptor-γ-modulating activity, was investigated against spatial working memory disturbances in mice subjected to chronic cerebral hypoperfusion.

Methods—Adult C57BL/6J male mice were subjected to bilateral common carotid artery stenosis using external microcoils. Mice received a daily oral administration of low-dose telmisartan (1 mg/kg per day), high-dose telmisartan (10 mg/kg per day), or vehicle with or without peroxisome proliferator-activated receptor-γ antagonist GW9662 (1 mg/kg per day) for all treatments for 30 days after bilateral common carotid artery stenosis. Cerebral mRNA expression of monocyte chemoattractant protein-1 and tumor necrosis factor-α was measured 30 days after bilateral common carotid artery stenosis, and postmortem brains were analyzed for demyelinating change with Klüver-Barrera staining and immunostained for glial, oxidative stress, and vascular endothelial cell markers. Spatial working memory was assessed by the Y-maze test.

Results—Mean systolic blood pressure and cerebral blood flow did not decrease with low-dose telmisartan but significantly decreased with high-dose telmisartan. Low-dose telmisartan significantly attenuated, but high-dose telmisartan provoked, spatial working memory impairment with glial activation, oligodendrocyte loss, and demyelinating change in the white matter. Such positive effects of low-dose telmisartan were partially offset by cotreatment with GW9662. Consistent with this, low-dose telmisartan reduced the degree of oxidative stress of vascular endothelial cells and the mRNA levels of monocyte chemoattractant protein-1 and tumor necrosis factor-α compared with vehicle.

Conclusions—Anti-inflammatory and antioxidative effects of telmisartan that were exerted in part by peroxisome proliferator-activated receptor-γ activation, but not its blood pressure-lowering effect, have protective roles against cognitive impairment and white matter damage after chronic cerebral hypoperfusion. (Stroke. 2010;41:1798-1806.)

Key Words: chronic cerebral hypoperfusion • oligovascular niche • oxidative stress • PPAR-γ • telmisartan

Drugs that target the renin–angiotensin system seem to have particular potential for prevention of dementias, including Alzheimer disease and vascular dementia. The Perindopril Protection Against Recurrent Stroke Study (PROGRESS) has suggested a protective effect of angiotensin-converting enzyme inhibitors on cognitive function in patients with stroke.1 Moreover, the Study on Cognition and Prognosis in the Elderly (SCOPE) trial demonstrated a positive effect of the angiotensin II Type 1 receptor blocker (ARB), candesartan, in a subgroup of elderly hypertensive patients with mild cognitive impairment.2 Notably, a prospective cohort analysis of 819,491 participants suggested that ARBs are associated with a significant reduction in the incidence and progression of dementia, even compared with angiotensin-converting enzyme inhibitors.3

No benefit was found in cognitive performance after administration of the ARB, telmisartan, at the subacute stage (within 15 days) after stroke in the Prevention Regimen for Effectively Avoiding Second Strokes (ProFESS) study.4 However, in vitro studies have suggested that telmisartan, the strongest peroxisome proliferator-activated receptor-γ (PPAR-γ) activator among ARBs,5 may protect oligodendrocytes and neurons through a reduction of brain inflammation through PPAR-γ activation and AT1 receptor blockade.
Telmisartan has structural similarities to the PPAR-γ ligand pioglitazone and thus could act as a partial agonist of PPAR-γ.5 PPAR-γ, one of the nuclear receptors, plays a critical role in a variety of biological processes, including angiogenesis, inflammation, oxidative stress, glucose metabolism, and adipogenesis.5 Moreover, PPAR-γ activation in the brain has been suggested as a protective effect against Alzheimer disease through its multifaceted effects, including anti-inflammation and amyloid-β clearance.6 Therefore, in the PROFESS study, excessive lowering of blood pressure (BP) in the period with cerebrovascular autoregulatory dysfunction may have affected the cerebral circulation and neuronal function, although other factors could also be involved.

The present study is therefore designed to explore the multifaceted effects of telmisartan on cognitive disturbances in a mouse model of vascular dementia by administering a hypotensive or a nonhypotensive dose of telmisartan. This model of chronic cerebral hypoperfusion, which is produced by placing microcoils bilaterally on the common carotid arteries, invariably exhibits glial activation, oxidative stress, inflammation, demyelinating change, and axonal loss in the white matter with resultant spatial working memory deficits.7,8 This in vivo system will help determine whether telmisartan affects vascular autoregulatory function and whether and how telmisartan exerts its protective effect against cognitive impairment related to white matter damage.

Materials and Methods

Experimental Protocol

The experimental protocol is shown in Figure 1. Nine-week-old male C57BL/6J mice (weighing 24 to 29 g; CLEA, Tokyo, Japan) were fed with the pelleted chow (MF) containing low-dose telmisartan (1 mg/kg per day), high-dose telmisartan (10 mg/kg per day), or vehicle with or without PPAR-γ antagonist GW9662 (1 mg/kg per day; Sigma-Aldrich) for all treatments, beginning from 7 days before the bilateral common carotid artery stenosis (BCAS) surgery until 30 days post-BCAS. Immediately after spatial working memory was assessed by the Y-maze test, mice were euthanized for histological and real-time reverse transcriptase-polymerase chain reaction examination 30 days post-BCAS.

Surgical Procedure of BCAS

Under anesthesia with halothane (2%), both common carotid arteries were exposed through a midline cervical incision, and a microcoil with an inner diameter of 0.18 mm was applied to the bilateral common carotid arteries. See Supplemental Method I for details (available at http://stroke.ahajournals.org).7,8

Systolic BP and Cerebral Blood Flow Measurements

Various doses of telmisartan (0 to 100 mg/kg per day) were administered and the BP measured for determining nonhypotensive and hypotensive doses of telmisartan. Then, in mice receiving the predetermined nonhypotensive or hypotensive dose of telmisartan or vehicle, systolic BP and cerebral blood flow (CBF) were monitored at 7 days before BCAS (before starting telmisartan treatment), immediately before BCAS, and 2 hours, 1 day, 3 days, 7 days, 14 days, and 30 days after BCAS. See Supplemental Method II for details.

Histochemical Evaluation of White Matter Lesions, Glial Activation, and Oxidative Stress

The mouse brains were analyzed for demyelinating change with Klüver-Barrera staining and immunostained for glial fibrillary acidic protein (a marker of astrocyte), ionized calcium binding adaptor molecule-1 (Iba-1; microglia), glutathione S-transferase-π (GST-π; oligodendrocyte), 8-hydroxy-deoxyguanosine (8-OHdG; oxidative stress), and CD31 (vascular endothelial cell). See Supplemental Method III for details.7

Quantitative Real-Time Reverse Transcriptase-Polymerase Chain Reaction

Cerebral mRNA levels of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) were assessed by quantitative real-time reverse transcriptase-polymerase chain reaction pre-BCAS and 30 days post-BCAS. Detailed procedures are described in Supplemental Method IV.
Y-Maze Test for Spatial Working Memory Assessment
Spatial working memory was assessed by the Y-maze test. The detail of the Y-maze test protocol is described in Supplemental Method V.

Blood Concentration of Telmisartan
See Supplemental Method VI for details.

Statistical Analysis
All values are expressed as means±SEM in the text and figures. One-way analysis of variance was used to evaluate significant differences among groups except when otherwise stated. When a statistically significant effect was found, a post hoc Tukey test or Tukey-Kramer test was performed to detect the difference between the groups. Temporal profiles of systolic BP and CBF were analyzed by 2-way repeated-measures analysis of variance followed by a post hoc Tukey test. Differences with \( P<0.05 \) were considered statistically significant in all statistical analyses used.

Results

Systolic BP and CBF After Telmisartan Administration
Treatment with telmisartan, ≤1 mg/kg per day, did not result in a significant reduction in BP, whereas treatment with telmisartan ≥3 mg/kg per day resulted in a significant reduction in BP (Figure 2A). CBF was not significantly reduced ≤1 mg/kg per day but began to decrease at 3 mg/kg per day (Figure 2B). A nonhypotensive dose of 1 mg/kg per day or a hypotensive dose of 10 mg/kg per day was subsequently administered. Temporal profiles of systolic BP and CBF were not affected by administration of a nonhypotensive dose of telmisartan or addition of GW9662 (Figure 2C–E). CBF gradually recovered after BCAS in mice with vehicle or a nonhypotensive dose of telmisartan but not in those with a hypotensive dose of telmisartan (Figure 2E).

The mortality rates were 10% at ≤1 mg/kg per day in the telmisartan-treated group after BCAS surgery. The mortality rate increased to 30% at 3 mg/kg per day in the telmisartan-treated group and 50% at 10 mg/kg per day in the telmisartan-treated group. Eighty percent at 50 or 100 mg/kg per day in the telmisartan-treated group died within 3 days post-BCAS.

Next, a hypotensive dose of telmisartan was examined to assess whether it ameliorated glial activation in BCAS-treated mice. In contrast to a nonhypotensive dose, a hypotensive dose of telmisartan caused substantial glial activation in the white matter (Figure 3; compare 3C and 3E and compare 3K and 3M). Cotreatment with GW9662 did not lead to additional glial changes in the white matter of mice with vehicle (Figure 3; compare 3A and 3B and compare 3I and 3J) or high-dose telmisartan (Figure 3; compare 3E and 3F and compare 3M and 3N).

Klüver-Barrera staining showed that white matter lesions were significantly attenuated in the nonhypotensive group compared with the vehicle group (Figure 4; compare 4A and 4C). Although patterns in oligodendrocytes arrangement could not be seen in the vehicle-treated mice (Figure 4A), alignment in a row formation could be seen in the group given a nonhypotensive dose of telmisartan (Figure 4C). Such effects of low-dose telmisartan were also partially offset by GW9662 (Figure 4; compare 4C and 4D) with significant differences (Figure 4G–H). In contrast, high-dose telmisartan did not attenuate white matter lesions (Figure 4; compare 4A and 4E). There were no significant histological differences between vehicle and high-dose telmisartan-treated mice with or without GW9662. In addition, administration of GW9662 had no effects on morphology of the white matter in sham-operated mice (data not shown), vehicle-treated, BCAS-operated mice (Figure 4; compare 4A and 4B), and high-dose telmisartan-treated, BCAS-operated mice (Figure 4; compare 4E and 4F). White matter lesion Grade 3 (disappearance of myelinated fibers) was only partially (approximately 10% of the white matter) observed in mice with vehicle or high-dose telmisartan.

In addition, the number of GST-\( \pi \)-positive oligodendrocytes of the vehicle-treated mice were significantly decreased in the white matter compared with that of mice treated with a nonhypotensive dose of telmisartan (Figure 4I–K). Telmisartan Attenuates mRNA Expression of Inflammatory Cytokines in Mouse Brain With Chronic Cerebral Hypoperfusion
Cerebral mRNA expression of inflammatory cytokines such as MCP-1 and TNF-\( \alpha \) was significantly increased after the BCAS but significantly attenuated by a nonhypotensive dose of telmisartan 30 days post-BCAS (Figure 4L–M).

Vascular Endothelial Oxidative Stress in Mouse Brain With Chronic Cerebral Hypoperfusion Was Ameliorated by Telmisartan
To further explore the antioxidative effect of telmisartan, 8-OHdG-positive vascular endothelial cells of the brain were assessed. The number of CD31-positive vascular endothelial cells positive for 8-OHdG was markedly reduced by a nonhypotensive dose of telmisartan (Figure 5; compare 5A and 5C). The difference was statistically significant as assessed by 8-OHdG/CD31-positive area (%; the percentage of 8-OHdG-positive area to CD31-positive area; Figure 5J). Such antioxidative effects of low-dose telmisartan were partially offset by cotreatment with GW9662 (Figure 5; compare 5A and 5D). By contrast, high-dose telmisartan showed an attenuated antioxidative effect in comparison to low-dose telmisartan (Figure 5; compare 5C and 5D).
Figure 2. Systolic BP (A) and CBF (B) in mice treated with various doses of telmisartan (n=10 each). Representative CBF images of the 6 groups of mice as assessed by laser speckle flowmetry 7 days before (Day -7) and immediately before (Day 0) BCAS and 30 days post-BCAS (Day 30; C). Temporal profiles of systolic BP (D) and CBF (E) of the 6 groups of mice (n=5 each). CBF was expressed as a percentage of baseline flow. F<sub>5,24</sub>=84.100 (D), F<sub>5,24</sub>=37.441 (E), *P<0.01 versus vehicle.
Figure 3. Representative images of immunohistochemistry for glial fibrillary acidic protein (GFAP; A–F) and Iba-1 (I–N) in the paramedian parts of the corpus callosum of the BCAS-operated mice treated with vehicle (A, I), vehicle + GW (B, J), Tel (Low; C, K), Tel (Low) + GW (D, L), Tel (High; E, M), and Tel (High) + GW (F, N) 30 days post-BCAS (n=7 each). Insets indicate enlarged images of astrocytes (A–F) and microglia (I–N). Scale bar, 100 μm. Histogram showing the density of GFAP-positive astrocytes (G–H) and Iba-1-positive microglia (O–P) in the corpus callosum (G, O) and anterior commissure (H, P) of the 6 groups of mice. Tel (Low) indicates low-dose telmisartan (1 mg/kg per day); Tel (High), high-dose telmisartan (10 mg/kg per day); GW, GW9662 (1 mg/kg per day).
Figure 4. Representative images of the Klüver-Barrera staining in the paramedian parts of the corpus callosum of the BCAS-operated mice treated with vehicle (A), vehicle + GW (B), Tel (Low; C), Tel (Low) + GW (D), Tel (High; E), and Tel (High) + GW (F) 30 days post-BCAS (n=7 each). Insets indicate enlarged images of oligodendrocytes. Histogram showing the grading of the white matter lesions of the 6 groups of mice (G–H; see Supplemental Method III for details). Representative images of immunohistochemistry for GST-π-positive oligodendrocytes in the medial parts of the corpus callosum of the BCAS-operated mice treated with vehicle (I) and Tel (Low; J) 30 days post-BCAS (n=7 each). Scale bar, 100 μm. Histogram showing the density of GST-π-positive oligodendrocytes (K) of the 2 groups of mice. Cerebral mRNA expressions of MCP-1 (L) and TNF-α (M) pre-BCAS and 30 days post-BCAS in the sham-operated or BCAS-operated mice treated with vehicle or Tel (Low; n=5 each). Tel (Low) indicates low-dose telmisartan (1 mg/kg per day); Tel (High), high-dose telmisartan (10 mg/kg per day); GW, GW9662 (1 mg/kg per day).
Compare 5A, 5C, and 5E). Cotreatment with GW9662 did not lead to additional histological changes in mice with vehicle (Figure 5; compare 5A and 5B) or high-dose telmisartan (Figure 5; compare 5E and 5F).

**Spatial Working Memory in Mice With Chronic Cerebral Hypoperfusion Was Restored by a Nonhypotensive Dose of Telmisartan**

Finally, we analyzed spatial working memory of BCAS mice by the Y-maze test as the final functional output. The percentage of alternation behaviors significantly decreased in vehicle-treated BCAS mice compared with the sham-operated mice but significantly increased in a nonhypotensive dose of telmisartan-treated mice. Such effects of low-dose telmisartan were partially offset by cotreatment with GW9662. Hypotensive doses of telmisartan-treated mice manifested in further impaired working memory (Figure 6A). There were no significant differences in the number of entries to each arm, which was considered to reflect locomotor activity, among the 5 groups (Figure 6B). These results suggest that a nonhypotensive dose of telmisartan, but not a hypotensive dose, improved spatial working memory of BCAS-operated mice.

**Blood Concentration of Telmisartan**

Plasma concentration after 1 mg/kg per day administration of telmisartan for 7 days was 142.86±14.85 ng/mL (n=4, values...
Effects, partially through PPAR-γ activation, telmisartan protected against white matter damage and is able to cross the blood–brain barrier. Telmisartan reduces NADPH oxidase activity; therefore, administration of telmisartan in such a low dose seems to decelerate the free radical system in the chronically hypoperfused mouse brain. However, a hypotensive dose of telmisartan substantially increased the degree of endothelial oxidative stress together with glial activation, white matter lesions, and spatial working memory deficits. Because mice with severe CBF reduction (approximately lower than 50% of the baseline level) died and were subsequently excluded from the analysis, such detrimental effects of high-dose telmisartan may even be underestimated. ARBs protect against ischemic cerebral injury, independently of BP. The benefit of normotension over hypotension lies in the absence of hypotension-induced aggravation of ischemic change. In the PROFESS study, lowering BP at the subacute phase after stroke (within 15 days) may have decreased CBF. The Acute Candesartan Cilexetil Therapy in Stroke Survivors (ACCESS) study indicated that candesartan treatment immediately after stroke, without significant lowering of BP, decreased the rate of recurrent stroke.

Therefore, the beneficial pleiotropic effect of telmisartan seems to be overwhelmed by BP-lowering if given in a high dose. Caution should therefore be exercised when lowering BP at an acute to subacute stage when cerebrovascular autoregulation is damaged. In clinical practice, appropriate timing and dose of telmisartan should be considered.

The effect of telmisartan on cognitive function was also assessed. The Y-maze tests showed that spatial working memory was significantly disrupted by chronic cerebral hypoperfusion. Nonhypotensive doses of telmisartan significantly attenuated the deterioration of spatial working memory together with histological improvement in the white matter. By contrast, hypotensive doses of telmisartan further aggravated spatial working memory together with histological deterioration. In this mouse model, pathological changes were restricted in the white matter only. The cerebral cortex and hippocampus, which are associated with spatial working memory, were not damaged in a relatively short period (for example, 30 days post-BCAS). Thus, telmisartan may have restored frontal–subcortical circuitry function, which is also associated with spatial working memory.

However, hippocampal changes appear at 3 months post-BCAS; it would therefore be of interest to extend the observation period to investigate effect of telmisartan on the gray matter in a future study.

Recently, it has been shown that cerebral endothelial cells secrete trophic factors that support the survival and proliferation of oligodendrocyte precursor cells. Such oligodendrocyte precursor cell-supportive phenomena in endothelial cells are mediated by Akt and Src signaling pathways. Noncytotoxic levels of oxidative stress downregulate the production through AT₁ receptor blockade. In addition, telmisartan suppresses MCP-1 expression through AT₁ receptor blockade and PPAR-γ activation. Genetic deletion of AT₁ receptor protects against damage due to brain ischemia. With its synergistic effects of AT₁ receptor blockade and PPAR-γ activation, telmisartan may exert multiple beneficial effects, including an antioxidative and anti-inflammatory effect, as shown in this study.

The nonhypotensive dose of telmisartan suppressed superoxide production from the vessel wall without lowering CBF. Telmisartan reduces NADPH oxidase activity; therefore, administration of telmisartan in such a low dose seems to decelerate the free radical system in the chronically hypoperfused mouse brain. However, a hypotensive dose of telmisartan substantially increased the degree of endothelial oxidative stress together with glial activation, white matter lesions, and spatial working memory deficits. Because mice with severe CBF reduction (approximately lower than 50% of the baseline level) died and were subsequently excluded from the analysis, such detrimental effects of high-dose telmisartan may even be underestimated. ARBs protect against ischemic cerebral injury, independently of BP. The benefit of normotension over hypotension lies in the absence of hypotension-induced aggravation of ischemic change. In the PROFESS study, lowering BP at the subacute phase after stroke (within 15 days) may have decreased CBF. The Acute Candesartan Cilexetil Therapy in Stroke Survivors (ACCESS) study indicated that candesartan treatment immediately after stroke, without significant lowering of BP, decreased the rate of recurrent stroke. Therefore, the beneficial pleiotropic effect of telmisartan seems to be overwhelmed by BP-lowering if given in a high dose. Caution should therefore be exercised when lowering BP at an acute to subacute stage when cerebrovascular autoregulation is damaged. In clinical practice, appropriate timing and dose of telmisartan should be considered.

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of trophic factors such as brain-derived neurotrophic factor and fibroblast growth factor and disrupt the ability of cerebral endothelial cells to support oligodendrocyte precursor cells. Furthermore, GST-π-positive oligodendrocytes were restored by low-dose telmisartan. These data suggest that a novel function of telmisartan is to maintain “oligovascular niche” by sustaining oligodendrocyte homeostasis in mammalian brain.

In conclusion, long-term AT1 receptor blockade and PPAR-γ activation with telmisartan should be considered as a novel therapeutic approach for protection from damage associated with chronic cerebral hypoperfusion or subcortical vascular dementia.

Acknowledgments
We thank Prof Kalaria for his excellent advice on this work and Dr Khundakar for his editorial assistance and comments. We are indebted to Ms Nakabayashi, Ms Gomibuchi, Ms Katsukawa, and Mr Kubota for their excellent technical assistance. Telmisartan was provided by Boehringer Ingelheim (Ingelheim, Germany). Boehringer Ingelheim provided no other support for this study, except supplying telmisartan.

Sources of Funding
This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Japanese Ministry of Education, Science and Culture (to J.T.) and a grant from the Suzuki Memorial Foundation (to M.I., J.T.).

Disclosures
None.

References
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Stroke. 2010;41:1798-1806; originally published online July 1, 2010; doi: 10.1161/STROKEAHA.110.583948

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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