Ameliorative Effects of Telmisartan on the Inflammatory Response and Impaired Spatial Memory in a Rat Model of Alzheimer’s Disease Incorporating Additional Cerebrovascular Disease Factors

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Telmisartan, an angiotensin type 1 receptor blocker, is used in the management of hypertension to control blood pressure. In addition, telmisartan has a partial agonistic effect on peroxisome proliferator activated receptor γ (PPARγ). Recently, the effects of telmisartan on spatial memory or the inflammatory response were monitored in a mouse model of Alzheimer’s disease (AD). However, to date, no studies have investigated the ameliorative effects of telmisartan on impaired spatial memory and the inflammatory response in an AD model incorporating additional cerebrovascular disease factors. In this study, we examined the effect of telmisartan on spatial memory impairment and the inflammatory response in a rat model of AD incorporating additional cerebrovascular disease factors. Rats were subjected to cerebral ischemia and an intracerebroventricular injection of oligomeric or aggregated amyloid-β (Aβ). Oral administration of telmisartan (0.3, 1, 3 mg/kg/d) seven days after ischemia and Aβ treatment resulted in better performance in the eight arm radial maze task in a dose-dependent manner. Telmisartan also reduced tumor necrosis factor α mRNA expression in the hippocampal region of rats with impaired spatial memory. These effects of telmisartan were antagonized by GW9662, an antagonist of PPARγ. These results suggest that telmisartan has ameliorative effects on the impairment of spatial memory in a rat model of AD incorporating additional cerebrovascular disease factors via its anti-inflammatory effect.

Key words telmisartan; tumor necrosis factor α; Alzheimer disease; inflammatory; rat

The epidemiological survey findings indicate that the Alzheimer’s disease (AD) rapidly progresses in case of which elderly people have a history of lifestyle-related diseases such as hypertension, lipid abnormality, diabetes and cerebrovascular disease (e.g., brain infarction). 1–5) The clinical report also indicates that the AD patients with a history of cerebrovascular disease exhibit a more rapid progression of dementia.6) Overall, 47% of demented participants in that clinical study had AD and/or additional brain infarcts, suggesting that the mixed form of such dementia may be very common in the elderly.

Based on the context of the above clinical studies, we have developed several AD-like animal models incorporating additional lifestyle-related disease factors, where the model animals in various clinical states were obtained by imposing the altering aggregate morphology of amyloid-β (Aβ) on naïve animals.7,8) Since these animal models reveal that administration of aggregated Aβ could induce neuronal cell death and spatial memory impairment, we have used them to evaluate the related-drug efficacy. Our pharmacological study indicates that the hypoxia treatment enhances Aβ-induced apoptosis in cultured hippocampal neurons.9)

Telmisartan, an angiotensin type 1 (AT1) receptor blocker that exerts a partial agonistic effect on the peroxisome proliferator-activated receptor γ (PPARγ), is generally employed to treat hypertension as well as metabolic syndrome. The PPARγ activation in the brain suppresses the inflammatory response in neuronal cells,10) endothelial cells,11) astrocytes, microglia,12) and also increases Aβ clearance.13) Therefore, telmisartan is expected to be a potential treatment option for AD. It has been reported that rosiglitazone, a PPARγ agonist, improves memory and learning in mice overexpressed a mutant form of the human amyloid precursor protein,14,15) and suppresses cognitive function decline in early forms of AD.16,17) Telmisartan is also reported to improve memory impairment of mice that had been intracerebroventricularly injected with Aβ.18,19)

Our previous studies confirmed the condition of single treatment of aggregated and oligomeric Aβ or only procedure of cerebral ischemia had no effects on spatial memory in radial maze task and hippocampal apoptosis.20,21) In this study, we examined the effect of telmisartan on spatial memory impairment in AD model rats incorporated cerebrovascular disease factor with pathological conditions induced by aggregated and oligomeric Aβ. We also studied the effect of telmisartan on the kinetics of tumor necrosis factor α (TNFα), an inflammation marker. Furthermore, the action of GW9662 (a PPARγ antagonist) on the effects of telmisartan was examined.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 250–300 g were obtained from KYUDO CO., LTD. (Saga, Japan). They were housed in groups of 4 to 5 per cage in a temperature-controlled room (23±2°C) with a relative humidity of 60±10%, and were maintained under a 12-h light–dark cycle. Food and water were available ad libitum except during the restricted feeding period. All procedures regarding animal care and use were carried out based on the regulations dictated by the Experimental Animal Care and Use Committee of Fukuoka
Preparation of Oligomeric Aβ and Aggregated Aβ

Aβ40 and Aβ42 peptides were purchased from Anaspec, Inc. (San Jose, CA, U.S.A.). Oligomeric Aβ was prepared as previously described.9) Aβ40 and Aβ42 peptides were dissolved in a N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES)-buffered solution to a final concentration of 10 µM. They were mixed at a molar ratio of 10:1 of Aβ40:Aβ42 and incubated at 37°C for 150 min (Oligomeric Aβ solution). Aggregated Aβ was prepared using Aβ42. Aβ42 peptide was dissolved in HEPES-buffered solution to a final concentration of 10 µM, and incubated at 37°C for 7 days.

Four-Vessel Transient Cerebral Ischemia

Four-vessel occlusion was performed as previously described.9) Briefly, rats were anesthetized with intraperitoneal injection of 50 mg/kg of sodium pentobarbital (Tokyo Kasei, Tokyo, Japan) and immobilized in a stereotaxic apparatus. The bilateral vertebral arteries were electrocauterized using a bipolar coagulator (MICRO-3D; Mizuho Industrial Co., Tokyo, Japan). The bilateral common carotid arteries were then exposed and threaded. On the next day, the common carotid arteries were compressed by clips, and cerebral circulation was interrupted for 10 min. Rats that did not exhibit a loss of their righting reflex during arterial occlusion were excluded from subsequent experiments.

Stereotaxic Procedure

The procedure was performed as previously described.9) Rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally (i.p.)) and placed in a stereotaxic frame (Narishige Scientific Instruments, Tokyo, Japan). For intracerebroventricular (i.c.v.) infusion, a guide cannula (0.71 mm in diameter, and 13 mm in length) was implanted bilaterally into the lateral cerebral ventricles at AP = −0.8 mm, L = ±1.3 mm, and H = 3.3 mm from bregma. The implanted cannula was anchored by two screws driven into the skull, fixed by dental acrylic cement, and then secured with a dummy cannula kept in place by a cap. After surgery, each rat was injected with penicillin in the hindquarter muscle (100000 U) and individually housed after operation.

Intracerebroventricular Injection of Aβ

Oligomeric Aβ or aggregated Aβ were injected intracerebroventricularly once daily for 7 d. In the Aβ-injected plus cerebral ischemia group, Aβ was injected daily for 7 d after CI. Aβ was injected bilaterally (oligomeric Aβ; 200 pmol/20 µL, aggregated Aβ; 600 pmol/20 µL) using an injection cannula (0.35±0.01 mm o.d., 0.17±0.02 mm i.d., and 14 mm length) connected by PE tubing (1.09 mm o.d., 0.38 mm i.d.; Intramedic; Becton Dickinson, San Jose, CA, U.S.A.) to a perfusion pump (CMA/100; Microdialysis AB, Stockholm, Sweden) driven at a rate of 1 µL/min. The first injection of Aβ was performed 1 h after cerebral ischemia.

Eight-Arm Radial Maze Task

Behavioral testing using an 8-arm radial maze task (Neuroscience Co., Tokyo, Japan) was conducted as previously reported.9) The maze consisted of a central platform (24 cm in diameter) with 8 arms that extended radially. Rats were allowed to visit each arm to eat 8 pellets in food cups located near the end of each arm. Each test animal was trained once per day to memorize the apparatus. The performance of the test animals in each trial was assessed using two parameters: number of correct choices in the initial 8 chosen arms and number of errors (defined as choosing arms that had already been visited). When the test animals made 7 or 8 correct choices and no more than one error in three successive sessions, they were deemed to have memorized the maze. In other words, the rats had acquired spatial memory of the 8-arm radial maze.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the hippocampus...
using TRIzol® reagent (Invitrogen; Carlsbad, CA, U.S.A.). After DNase treatment using the TURBO DNA-free Kit (Invitrogen; Carlsbad, CA, U.S.A.), 1 µg RNA from each sample was reverse-transcribed using ReverTra Ace® qPCR RT Kit (TOYOBO; Osaka, Japan). Generated cDNA was amplified using THUNDERBIRD SYBR® qPCR Mix (TOYOBO; Osaka, Japan) with the following primer sets: glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward 5'-GCTGCCAAGGCTGTGGGCAGAGC-3'; reverse 5'-GCGCTTACCACC TTC-3'; TNF-α: forward 5'-CGAGTGACAAGGCCCCGTAGCC-3'; reverse 5'-GACGGTGTTGGTAGGAGGACAC-3. Relative TNF-α mRNA expression was determined using the 2^ΔΔCT method, with standardization against GAPDH and normalization to the sham control.

Materials and Treatment Telmisartan was a gift from Nippon Boehringer Ingelheim (Tokyo, Japan). The drug was suspended in 0.5% (v/v) hydroxyethylcellulose (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The administration of GW9662 was performed as previously described.22) GW9662 (Cayman Chemical; Ann Arbor, MI, U.S.A.) was dissolved in dimethylsulfoxide (Wako Pure Chemical, Osaka, Japan) (25% (w/v)) and intracerebroventriculally administered at dose of 18 nmol/20 µL just before oral administration of telmisartan. The guide cannula (inside diameter: 22 gauge, length: 13 mm) for intracerebroventricularly administration was implanted at the following coordinates: A: 0.8 mm; L: 1.3 mm; H: 3.8 mm.

Statistical Analysis Results are expressed as the mean±S.E.M. Statistical significance within each group was estimated using the F-test followed by the Mann–Whitney’s U-test. When the experimental series involved more than two groups, one-way analysis of variance was used, with a post-hoc Tukey’s test. p<0.05 was considered statistically significant.

RESULTS

Transient cerebral ischemia (CI) and i.c.v. infusion of oligomeric Aβ significantly reduced the number of correct choices and increased the number of errors compared with sham-operated rats in the 8-arm radial maze task. Telmisartan (3 mg/kg) did not have any effect on the reduction of the number of correct choices and the increment of the number of errors induced by cerebral ischemia and infusion of oligomeric Aβ (Fig. 1).

Transient CI and i.c.v. infusion of aggregated Aβ significantly reduced the number of correct choices and increased the number of errors compared with sham-operated rats in the 8-arm radial maze task. Post-ischemic administrations of telmisartan (3 mg/kg) for 7 d significantly increase the number of correct choices and decreased the number of errors in the 8-arm radial maze in CI+Aβ-aggregate treated rats (Fig. 2). These improvements by telmisartan completely disappeared with the concurrent use of the PPARγ antagonist, GW9662 (Fig. 3).

Hippocampal TNFα mRNA expression after CI+Aβ-aggregate treatment was shown in Fig. 4. After CI+Aβ-aggregate treatment, TNFα mRNA expression in the dorsal hippocampus was highest at 6 h after ischemia and subsequently decreased.

Hippocampal TNFα mRNA expression after treatment of telmisartan and additional treatment of GW9662 in CI+Aβ-aggregate rats was shown in Fig. 5. After transient CI, a single i.c.v. dose of aggregated Aβ was given to mice. When telmisartan (3 mg/kg) was orally administered, TNFα mRNA expression, which was observed to increase at 6 h after ischemia, significantly decreased. This effect of telmisartan disappeared with the concurrent use of GW9662.

Fig. 2. Effect of Telmisartan on Spatial Memory Impairment Induced by the Combination of Aggregated Aβ and Cerebral Ischemia

The test was performed 1h after the last aggregated Aβ injection. The number of correct choices is presented on the left panel and the number of errors is presented on the right panel in rats receiving aggregated Aβ and cerebral ischemia with or without telmisartan treatment. The last treatment of telmisartan was performed 60min prior to the test. Values are expressed as the means±S.E.M. n=7–8 rats. **p<0.01 vs. Sham group, Mann–Whitney’s U-test. †p<0.05 vs. Vehicle group, one way ANOVA post-hoc Tukey’s test.
The mechanisms of Alzheimer’s disease (AD) cannot be simply explained by the basis of amyloid-β (Aβ) accumulation. The deterioration of the metabolic system including the degradation and clearance of Aβ is likely to be involved. Animals in various clinical states were obtained by altering the aggregate morphology of Aβ administered in the animal models. The CI+Aβ-aggregate rats presented with spatial memory impairment, due to marked neuronal cell death in the hippocampal CA1 region, preceded by decreased acetylcholine release in the hippocampal region. Meanwhile, administration of oligomeric Aβ does not induce neuronal cell death, but cause spatial memory impairment. The oligomeric Aβ-induced spatial memory impairment is accompanied by the dysfunction of dynamin-1 which is a protein involved in acetylcholine release machinery. Thus, we consider the oligomeric Aβ-induced spatial memory impairment to be model of mild cognitive impairment. The results of the 8-arm radial maze task indicate that telmisartan alleviated spatial memory impairment in CI+Aβ-aggregate-treated rats. The ameliorative effect of telmisartan on spatial memory impairment via the reduction of neuronal cell death is indicated from the following findings: reduction of neuronal cell death in the hippocampal CA1 region and improvement of impaired spatial memory in rats with repeated cerebral ischemia (cerebrovascular dementia model); lack of improvement in spatial memory impairment in CI+Aβ-oligomer rats that did not exhibit hippocampal neuronal cell death.

PPARγ is reported to suppress the expression of TNFα, interleukin 1β (IL-1β), and other inflammation-related molecules. Although detailed mechanism underlying the gene expression suppression is not well known, it seems to be that these inflammation-related genes are located in downstream of nuclear factor κB (NF-κB) signaling; therefore, the suppressive target of PPARγ is considered to be at the level of NF-κB gene expression. NF-κB is one of the transcription factors that play a central role in immunological responses, and is involved in a number of physiological phenomena including acute/chronic inflammatory responses, cell growth, and neuronal cell death. By the same token, the involvement of PPARγ in these physiological phenomena is also indicated. Activated microglia that is responsible for brain inflammatory responses accumulates in AD lesions, and the levels of pro-inflammatory cytokines including TNFα, IL-1β, and IL-6 are increased. According to recent report, an inflammatory mediator is produced from microglia and macrophages following the intracerebroventricular (i.c.v.) infusion of Aβ, which is believed to induce AD-like phenotypes in rats. Furthermore, the epidemiological survey shows that rheumatic patients who took non-steroidal anti-inflammatory drugs (NSAIDs) for extended periods of time have lower risks of developing AD. Accordingly, telmisartan is expected to reduce the risk of AD owing to its inflammation-suppressing effect. In fact, telmisartan was reported to reduce the expression of TNFα mRNA in mice. Our study also demonstrated that the expression of TNFα mRNA was elevated 6h after ischemia in the brain of CI+Aβ-aggregate rats, and that telmisartan suppressed the increased post-ischemic expression of TNFα mRNA. Furthermore, GW9662, a PPARγ antagonist, counteracted the ameliorative effect of telmisartan on spatial memory impairment, and increased the expression of TNFα mRNA. Therefore, our
study suggests that telmisartan improve in spatial memory impairment in CI+Aβ-aggregate rats by suppressing TNFα-induced neuronal cell death via PPARγ. In this study, the treatment of GW9662 was according to previous study.22) Intracerebroventricular injection of GW9662 blocked the ameliorative effect of telmisartan on impaired spatial memory in rats treated with repeated cerebral ischemia. After intracerebroventricular injection of GW9662, any adverse symptoms such as staggering gate or sedation was not observed. Other reports applied the higher dose of GW9662 than that of this study. Therefore, the dose of GW9662 in this study is reasonable to antagonize the effects of telmisartan.

The role of the AT1 receptor cannot be ruled out as a contributing factor to the ameliorative effect of telmisartan on spatial memory impairment. AT1 is known to be broadly distributed in the brain. Inhibition of AT1 suppresses ischemia-induced degeneration/dropout of mitochondria and oxidative stress. The expression levels of nicotinamide phosphoribosyl transferase (Nampt), sirtuin 3 (Sirt3) and other prosurvival genes are increased by AT1 suppression.30) While PPARγ is expressed in the fetal brain as a factor involved in cellular development, its expression is quite limited in the healthy mature brain.31) However, it has been suggested that while the PPARγ pathway is necessary for the growth of undifferentiated neural stem cells, it also plays an important role in maintaining the undifferentiated state.32) Furthermore, a recent report on central PPARγ showed that cerebral ischemia increases the expression of PPARγ.33) Considering that an ameliorative effect on spatial memory impairment was demonstrated by both the PPARγ full agonist pioglitazone and the PPARγ partial agonist telmisartan, and that the ameliorative effect of telmisartan on spatial memory impairment in CI+Aβ-aggregate rats was almost totally eliminated by the PPARγ antagonist GW9662, we believe that the role of telmisartan as a PPARγ partial agonist may be greater than that of AT1 in mediating the ameliorative effect of telmisartan on spatial memory impairment. In fact, telmisartan reduced neuronal cell death in the hippocampal CA1 region and alleviated spatial memory impairment in rats with repeated CI, which is a model of cerebrovascular dementia.22)

In our study, the inhibition of Aβ-toxicity by telmisartan possibly ameliorated spatial memory impairment in CI+Aβ-aggregate rats. PPARγ activation may reduce Aβ accumulation based on the following findings: PPARγ activation by...
telmisartan suppressed the expression of the proteolytic enzyme β-secretase, which produces Aβ from the beta-amyloid precursor protein (APP)\(^{26}\); suppression of cerebral blood flow lowering in AD model animals inhibited the hypometabolism of Aβ\(^{15,35}\), and acceleration of APP ubiquitination renders it easily degradable.\(^{36}\) It was also revealed that PPAR-γ suppresses microglial activation \(v\)ia Aβ accumulation in the brain\(^{37}\) and the associated expression of inflammatory cytokines.\(^{38}\) Therefore, the effects of telmisartan on Aβ kinetics in the brains of our model rats require further investigation.

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