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Original article

Telmisartan protects against cognitive decline via up-regulation of brain-derived neurotrophic factor/tropomyosin-related kinase B in hippocampus of hypertensive rats

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ABSTRACT

Background and purpose: Cognitive decline may occur as a result of hypertension, and is dependent on the function of hippocampus. Brain-derived neurotrophic factor (BDNF) mediated by angiotensin II-induced oxidative stress protects against cell death in hippocampus. Angiotensin II receptor blocker (ARB), candesartan, activates BDNF in the hippocampus. Furthermore, peroxisome proliferator-activated receptor (PPAR)-gamma activation in the brain prevents brain damage. Telmisartan, a unique ARB with PPAR-gamma stimulating activity, protects against cognitive decline partly because of PPAR-gamma activation. The aim of the present study was to determine whether telmisartan protects against cognitive decline via up-regulation of BDNF and its receptor tropomyosin-related kinase B (TrkB) in the hippocampus of hypertensive rats, partly because of PPAR-gamma activation.

Methods and results: We divided stroke-prone spontaneously hypertensive rats (SHRSPs), as hypertensive and vascular dementia model rats, into five groups, telmisartan-treated (TLM), TLM + GW9662, a PPAR-gamma inhibitor, -treated (T + G), GW9662-treated (GW), TLM + ANA-12, a TrkB antagonist, -treated (T + A), and vehicle-treated SHRSPs (VEH). After the treatment for 28 days, systolic blood pressure did not change in all groups. However, BDNF expression in the hippocampus was significantly higher in TLM than in VEH to a greater extent than in T + G. Cognitive performance was significantly higher in TLM than in VEH to a greater extent than in T + G, and was not different between T + A, GW, and VEH.

Conclusion: Telmisartan protects against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus of SHRSPs, partly because of PPAR-gamma activation independent of blood pressure-lowering effect.

Introduction

One of the important organ damages related to hypertension is cognitive decline. In the brain, angiotensin II contributes to the physiological regulation of many different functions, including cerebral circulation, integrity of the blood–brain barrier, central sympathetic activity, hormonal production and release, response to stress, behavior, and cognition [1–5]. In the treatments for hypertension, angiotensin II type1 receptor (AT_1R) blockers (ARB) are widely used [6]. A previous clinical study demonstrated that antihypertensive drugs that act via the renin–angiotensin system have potential in preventing, delaying, or decelerating the onset and progression of cognitive decline in hypertensive patients [7]. In the treatments for hypertension, cognition should be focused as a target of the antihypertensive treatment. Among ARBs, telmisartan has a beneficial effect in rats treated with repeated cerebral ischemia [8,9], Alzheimer model [10,11], diabetic model [12], and coronary plaque vulnerability [13]. However, no benefit was found in cognitive performance after administration of telmisartan after stroke [14]. In ONTARGET and TRANSCEND, telmisartan did not provide positive effects on cognitive function [15]. The mechanisms of the protection against cognitive decline in cerebral ischemia by telmisartan should be discussed further. Telmisartan is a unique ARB with a partial peroxisome proliferator-activated receptor (PPAR)-gamma agonistic property in its antihypertensive effect [16]. Anti-inflammatory and anti-oxidant effects of telmisartan that were exerted in part by PPAR-gamma activation, but not its

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blood pressure-lowering effect, have protective roles against cognitive decline in cerebral ischemia [8,9]. PPAR-gamma activation is reported to reduce oxidative stress and inflammatory response in the vasculature and adipose tissue [17], and PPAR-gamma activation in the brain has been reported to prevent brain damage via anti-inflammatory effects in neurons [18].

Previous studies have suggested that the underlying mechanisms of the beneficial effect of ARBs in stroke may not only be the consequence of improved hemodynamics and vascular function, but may also involve a blood pressure-independent element of neuroprotection [19–22]. In the brain, brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B (TrkB) are known to be involved in the protective mechanisms against stress and cell death as an antioxidant [23–26]. Angiotensin II induces superoxide-dependent down-regulation of BDNF via phosphorylation of cAMP response element binding protein [27]. Candesartan at sub-hypotensive and renin–angiotensin system blocking dose affords neuroprotection after focal ischemia, associated with increased activity of BDNF [28]. Telmisartan improves memory impairment and reduces neural apoptosis in hippocampus via a PPAR-gamma-dependent anti-apoptotic mechanism in rats with repeated cerebral ischemia [8]. However, it has not been determined whether telmisartan has protective effects on cognitive decline via up-regulation of BDNF/TrkB in the hippocampus.

Combined with these previous studies, we had the hypothesis that the beneficial effects of telmisartan on cognition are not only because of its established effect of antihypertensive and systemic blockade of AT_1R but also because of the benefits on BDNF in the hippocampus via PPAR-gamma agonistic effect in hypertension. The aim of the present study was to determine whether telmisartan protects against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus of stroke-prone spontaneously hypertensive rats (SHRSPs) as hypertensive and vascular dementia model rats [29], partly because of PPAR-gamma activation. Previous studies have demonstrated that ARBs have benefits on brain damage and vascular inflammation in SHRSPs [30–32], as well as organ damage in spontaneously hypertensive rats [33]. Telmisartan also has anti-oxidant effects in vasculature [34] and brain [35] of SHRSPs. We divided SHRSPs into five groups, telmisartan-treated (TLM), TLM + GW9662, a PPAR-gamma antagonist, -treated (T + G), GW9662-treated (GW), TLM + N-[2-[[(hexahydro-2-oxo-1H-azepin-3-yl) amino] carbonyl] phenyl]-benzothiophene-2-carboxamide (ANA-12), a TrkB antagonist, -treated (T + A), and vehicle-treated SHRSPs (VEH). Cognitive function was assessed by the Morris water maze test, which has been widely used as a test of spatial memory and cognition [36].

Methods

Animals

This study was reviewed and approved by the committee on ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments of Kyushu University. Male SHRSPs (12–14 weeks), weighing 350–425 g and fed standard feed were used (SLC Japan, Hamamatsu, Japan). They were housed individually in a temperature-controlled room (22–23 °C) with a 12-h/12-h light-dark cycle (lights on at 7:00 AM). We divided SHRSPs into 5 groups: TLM, T + G, T + A, GW, and VEH ($n = 5$ for each). Systolic blood pressure and heart rate were measured daily using the tail-cuff method (BP-98 A; Softron, Tokyo, Japan).

Oral administration of drugs

SHRSPs were treated for 4 weeks. TLM group was administered telmisartan (1 mg/kg/day, Sigma Aldrich, St. Louis, MO, USA). GW group was administered GW9662 (1 mg/kg/day, Sigma Aldrich). T + G group was administered telmisartan (1 mg/kg/day) plus GW9662 (1 mg/kg/day). T + A group was administered telmisartan (1 mg/kg/day) plus ANA-12 (0.5 mg/kg/day, Sigma Aldrich). VEH group was administered 0.5% methylcellulose. All drugs were dissolved in 0.5% methylcellulose and administered by gastric gavage every day. The dose of telmisartan was selected as a low dose and non-depressor dose [37,38]. The dose of GW9662 was according to the previous studies examining the partial effect of telmisartan on PPAR-gamma activation [9,37]. The dose of ANA-12 was determined to blockade BDNF according to a previous study [39].

Western blotting analysis

To obtain the hippocampus tissues, the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg IP) and perfused transcardially with PBS (150 mol/L NaCl, 3 mmol/L KCl, and 5 nmol/L phosphate; pH 7.4, 4 °C). The brains were removed quickly, and the hippocampus tissues obtained according to a rat brain atlas were homogenized and sonicated in a lysing buffer containing 40 mmol/L HEPES, 1% Triton X-100, 10% glycerol, and 1 mmol/L phenylmethanesulfonyl fluoride. The tissue lysate was centrifuged at 6000 rpm for 5 min at 4 °C with a microcentrifuge. The lysate was collected, and protein concentration was determined with a BCA protein assay kit (Pierce, Rockford, IL, USA). An aliquot of 20 μg of protein from each sample was separated on 12% SDS-polyacrylamide gel. Proteins were subsequently transferred onto polyvinylidene difluoride membranes (Immobilon-P membrane; Millipore, Billarica, MA, USA). Membranes were incubated for 2 h with a rabbit polyclonal antiserum against BDNF (1:1000; Abcam, Cambridge, UK) or α-tubulin (1:1000; Cell Signaling, Danvers, MA, USA). Membranes were then washed and incubated with a horseradish peroxidase–conjugated horse anti-mouse IgG antibody (1:10,000) for 40 min. Immunoreactivity was detected by enhanced chemiluminescence autoradiography (plus Western blotting detection kit; GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and was expressed as the ratio to α-tubulin protein.

Analysis of cognitive function

Spatial learning and memory function of the rats were investigated with the Morris water maze test in a circular pool filled with water at a temperature of 25.0 ± 1 °C [36]. In the hidden platform test, a transparent platform was submerged 1 cm below the water level. Swimming paths were tracked with a camera fixed on the ceiling of the room and stored in a computer. All the procedures of the Morris water maze were performed for 7 days. A pre-training session was carried out at day 0, in which animals were given 60 s free swimming without the platform. In the hidden-platform test for 4 days, the rats were given 2 trials (1 session) on day 1 and 4 trials (2 sessions) per day on days 2, 3, and 4. The initial trial interval was about 30 min and the inter-session interval was 2 h. During each trial, the rats were released from four pseudo-randomly assigned starting points and allowed to swim for 60 s. After mounting the platform, the rats were allowed to remain there for 15 s, and were then placed in the home cage until the start of the next trial. If a rat was unable to find the platform within 60 s, it was guided to the platform and allowed to rest on the platform for 15 s. Probe trials were performed at day 5. In the probe trial, the hidden platform was removed and the rats was released from the right quadrant and allowed to swim freely for 60 s. The time spent in the target