Neurovascular Protection by Telmisartan via Reducing Neuroinflammation in Stroke-Resistant Spontaneously Hypertensive Rat Brain after Ischemic Stroke

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Telmisartan is a highly lipid-soluble angiotensin receptor blocker (ARB), which improves insulin sensitivity and reduces triglyceride levels and, thus, is called metabo-sartan. We examined the effects of telmisartan on neurovascular unit (*N*-acetylglucosamine oligomer [NAGO], collagen IV, and glial fibrillary acidic protein [GFAP]) and neuroinflammation (matrix metalloproteinase-9 [MMP-9] and inflammasome) in brain of stroke-resistant spontaneously hypertensive rat (SHR-SR). At 12 weeks of age, SHR-SR received transient middle cerebral artery occlusion (tMCAO) for 90 minutes and were divided into the following 3 groups, that is, vehicle group, low-dose telmisartan group (.3 mg/kg/d), and high-dose telmisartan group (3 mg/kg/d, postoral). Immunohistologic analysis at ages 6, 12, and 18 months showed progressive decreases of NAGO-positive endothelium and collagen IV–positive basement membrane and progressive increases of MMP-9–positive neurons, GFAP-positive astrocytes, and NLRP3-positive inflammasome in the cerebral cortex of vehicle group. Low-dose telmisartan reduced such changes without lowering blood pressure (BP), and high-dose telmisartan further improved such changes with lowering BP. The present findings suggest that a persistent hypertension caused a long-lasting inflammation after tMCAO in SHR-SR, which accelerated neurovascular disruption and emergent inflammasome, and that telmisartan greatly reduced such inflammation and protected the neurovascular unit via its pleiotropic effects in living hypertensive rat brain after ischemic stroke. **Key Words:** Spontaneously hypertensive rat—neurovascular unit—inflammasome—telmisartan—Alzheimer disease.

Introduction

Stroke-resistant spontaneously hypertensive rats (SHR-SRs) and stroke-prone SHR are the most widely used animal model of essential hypertension, sharing several similarities with human essential hypertension. The SHR also represents a model of multiple metabolic disorders similar to the human metabolic syndrome.[1-3]

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Previous studies demonstrated that the SHR developed insulin resistance[4] and several risk factors for Alzheimer disease (AD).[5]

Some clinical trials reported that the effect of angiotensin II type 1 (AT1) receptor blockers showed primary and secondary preventions of cerebral infarction.[6-9] Treatment of angiotensin receptor blockers (ARBs) has been shown to reduce stroke volume and improve neurologic function through their blockade of the renin–angiotensin system in experimental stroke model.[10-15] Although the antihypertensive effect of ARBs are one of the mechanisms of preventing cerebral infarction, ARBs have a blood pressure–independent neuroprotection with reducing oxidative stress and upregulating angiotensin II type 2 signaling.[16-23]

Telmisartan is a highly lipid-soluble AT1 receptor blocker[24,25] and is a partial agonistic effect on peroxisome proliferator–activated receptor gamma, which improves insulin sensitivity and reduces triglyceride levels[26-28] and, thus, is called metabo-sartan to exert a special protective effect for both acute brain damage and chronic progressive dementia.[29,30] There has been, however, no study on the long-term effects of telmisartan on both protection of the neurovascular unit and reduction of inflammation after stroke. We, therefore, examined the effects of telmisartan on SHR-SR with transient middle cerebral artery occlusion (tMCAO) by both doses with effect of antimetabolic mechanism, that is, a low dose without lowering blood pressure and a high dose with lowering hypertension.

Materials and Methods

Animals

Seven-week-old male SHR-SRs were provided from Disease Model Cooperative Research Association (Kyoto, Japan) and placed on a basal diet. Animals were maintained for at least 7 days before the experiment in a temperature-regulated room (23°C-25°C) on a 12-hour light/dark cycle. The rats were fasted but allowed free access to water overnight before surgery.

Ischemia/Reperfusion Model

Transient focal cerebral ischemia was induced in SHR-SR by right MCAO at 12 weeks of age (body weight 260-280 g). Briefly, the SHR-SR were anesthetized by inhalation of a 69%/30% (vol/vol) mixture of nitrous oxide/oxygen with 1% halothane using a face mask. A midline neck incision was made, and the right common carotid artery was exposed. The right MCA was occluded by insertion of 4-0 surgical nylon thread with a silicone coating through the common carotid artery immediately after ligation of the ipsilateral common and external carotid arteries as described previously.[31] Using this technique, the tip of the thread occludes the origin of the right MCA. During these procedures, body temperature was monitored with a rectal probe and was maintained at 37 ± .3°C using a heating pad. The surgical incision was then closed, and the animals were allowed to recover at room temperature. After 90 minutes of tMCAO, the suture was removed to restore blood flow (reperfusion). Sham control animals were treated with cervical surgery but without insertion of the nylon thread. The animals were kept at ambient temperature until sampling, with free access to water and food.

Drug Preparation

On the day of tMCAO at 12 weeks, the above SHR-SR (n = 51) were divided into the following 3 groups after tMCAO, that is, SHR-SR vehicle group (SHR/Ve, n = 19), SHR-SR low-dose telmisartan group in which the blood pressure did not fall (SHR/Low, n = 16), and SHR-SR high-dose telmisartan group in which the blood pressure significantly fell by 30 mm Hg or more (SHR/High, n = 16), receiving daily oral doses of .5% methylcellulose (MC, 0.1 mL) only (SHR/Ve group), .5% MC plus low-dose telmisartan (0.3 mg/kg/d), or .5% MC plus high-dose telmisartan (3 mg/kg/d) for the subsequent 3, 9, and 15 months until sacrifice by oral gavage, respectively. The dose of telmisartan was determined as previously described.[32,33] Telmisartan was provided by Boehringer Ingelheim (Ingelheim am Rhein, Germany) and was given to the 2 rat groups as a suspension with 5% MC in 0.1 mL water every day.

At ages 6, 12, or 18 months, the rats were transcardially perfused with 5 U/mL chilled heparinized saline followed by 4% paraformaldehyde in phosphate buffer (pH 7.6) under deep anesthesia with pentobarbital (20 mg/250 g rat). After decapitaion, their brains were removed. All experimental procedures were approved by the Animal Committee of the Graduate School of Medicine and Dentistry, Okayama University. The present study focused on neurovascular unit components and inflammasome in a whole project in the examination of effect of telmisartan on SHR-SR.

Immunohistochemistry

After the removal, the brains were immersed and fixed in 4% paraformaldehyde with .1 M phosphate buffer (pH 7.6) for 8 hours, embedded in paraffin, and 5-μm-thick sections were prepared for subsequent immunostaining. For *N*-acetylglucosamine oligomer (NAGO), which was used as the specific endothelial cell marker,[34] collagen IV, matrix metalloproteinase-9 (MMP-9), glial fibrillary acidic protein (GFAP), and nucleotide-binding and oligomerization domain–like receptor family, pyrin domain–containing 3 (NLRP3) inflammasome, the brain sections were pretreated by heating them 3 times in a 500-W microwave for 5 minutes in 10 mM (pH 6.0) citric acid buffer. These pretreated sections were then immersed in