Telmisartan Reduces Progressive Accumulation of Cellular Amyloid Beta and Phosphorylated Tau with Inflammatory Responses in Aged Spontaneously Hypertensive Stroke Resistant Rat

Tomoko Kurata, MD, Violeta Lukic, PhD, Miki Kozuki, BS, Daisuke Wada, BS, Kazunori Miyazaki, PhD, Nobutoshi Morimoto, MD, PhD, Yasuyuki Ohta, MD, PhD, Kentaro Deguchi, MD, Yoshio Ikeda, MD, PhD, Tatsushi Kamiya, MD, PhD, and Koji Abe, MD, PhD

Background: In addition to reducing the level of blood pressure (BP), telmisartan was expected to show the long-term neuroprotective effects preventing accumulation of cellular amyloid beta peptide (Aβ) and phosphorylated tau (pτ) by ameliorating neuroinflammation. *Methods:* We examined effects of telmisartan on cellular Aβ and pτ with inflammatory responses in the brain of a spontaneously hypertensive stroke resistant (SHR-SR) rat by giving either telmisartan at 0 (vehicle), .3 mg/kg/day or 3 mg/kg/day, orally, from 3 months of age and performed immunohistologic analysis at 6, 12, and 18 months. Compared with normotensive Wistar rats, numbers of Aβ- and pτ-positive neurons in the cerebral cortex progressively increased with age until 18 months in the SHR-SR rats, as did the numbers of ionized calcium-binding adapter molecule 1 (Iba-1)-positive microglia, tumor necrosis factor alpha (TNF-α)-positive neurons, and monocyte chemotactic protein 1 (MCP-1)-positive neurons. *Results:* Low-dose telmisartan significantly decreased the numbers of Aβ- and pτ-positive neuron as well as the numbers of TNF-α-positive neurons, Iba-1-positive microglia, and MCP-1-positive neurons at 6, 12, and 18 months. High-dose telmisartan reduced BP and showed a further reduction of cellular Aβ and pτ. *Conclusions:* The present study suggests that accumulation of cellular Aβ and pτ and the inflammatory responses were decreased via improving metabolic syndrome with low-dose telmisartan and improving both metabolic syndrome and hypertension with high-dose telmisartan. **Key Words:** Alzheimer's disease—spontaneously hypertensive rat—telmisartan—inflammation.

From the Department of Neurology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan.

Received January 22, 2014; revision received May 20, 2014; accepted May 29, 2014.

This work was partly supported by a Grant-in-Aid for Scientific Research (B) 21390267 from the Research Committee of CNS Degenerative Diseases; the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by Grants-in-Aid from Aoki M, Matsuoka Y, Mizusawa H, Nakano I, Nishizawa M, and Sasaki H from the Ministry of Health, Labour and Welfare of Japan.

Address correspondence to Koji Abe, MD, PhD, Department of Neurology, Okayama University Graduate School of Medicine, Dentistry and Pharmacy, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. E-mail: toko11@cc.okayama-u.ac.jp.

1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2014.05.023

Introduction

Alzheimer disease (AD) is the most common neurodegenerative dementia in the aged and is characterized neuropathologically by abnormal accumulations of amyloid plaques and neurofibrillary tangles (NFTs) throughout the cerebrocortical and limbic regions. Cognitive dysfunction of AD is widely explained by a progressive synaptic dysfunction and neurodegeneration initiated by amyloid beta peptide (Aβ) and phosphorylated tau (pτ), a principal component of NFTs.

Recent studies suggested that hypertension is an important risk factor of dementia, including AD[1,2] and vascular dementia,[3,4] which is associated with amyloid pathology via promoting inflammatory responses.[5,6] The spontaneously hypertensive (SHR) rat develops progressive neurobehavioral impairment[7] and several AD risk factors such as chronic hypertension,[8] metabolic syndrome with lipid dysfunction[9,10] and insulin resistance,[11] and immune alterations[12] during the long period. In fact, SHR rat displayed characteristic AD pathologies such as pτ, NFTs, and neuroinflammation.[13,14]

Various antihypertensive drugs prevented a cognitive decline or an incidence of dementia,[15,16] among which angiotensin receptor blockers (ARBs) showed superior effects on cognitive function[17] with the pleiotropic effects against neuroinflammation and vascular remodeling.[18,19] Telmisartan is a highly selective angiotensin (AT) type 1 antagonist of AT-2 receptor with high lipid solubility,[20,21] thus exerting a special protective effect for both acute brain damage and chronic progressive dementia. There has been, however, no study on the long-term effects of telmisartan in relation to chronic progressive dementia model of rats. We therefore examined the effects of telmisartan for accumulation of Aβ and pτ, which was considered an important part of Alzheimer pathology in SHR-SR rat, showing hypertension and metabolic dysfunction during long period, in both doses, that is, low dose just for improving metabolic syndrome of rats, and high dose for improving both metabolic syndrome and hypertension.

Materials and Methods

Animals and Drug Preparation

Seven-week-old male Wistar rats and stroke resistant spontaneously hypertensive (SHR-SR) rats were provided from Disease Model Cooperative Research Association (Kyoto, Japan) and placed on a basal diet.

When the rats reached 3 months of age, the previously mentioned Wistar rats (N = 20) were started on a daily dose of .5% methylcellulose (MC) in 0.1 mL water by oral gavage as normotensive control group for the subsequent 3-18 months, and the previously mentioned SHR-SR rats (N = 54) were divided into the following 3 treatment groups, that is, SHR-SR vehicle group (SHR/Ve, n = 17), SHR-SR low-dose telmisartan group in which the blood pressure (BP) did not fall significantly (SHR/low, n = 19), and SHR-SR high-dose telmisartan group in which the BP fell by 30 mm Hg or more (SHR/high, n = 18), receiving daily oral doses of .5% MC only (SHR/Ve), .5% MC plus low-dose telmisartan (0.3 mg/kg/day), or .5% MC plus high-dose telmisartan (3 mg/kg/day) for the subsequent 3-18 months by oral gavage, respectively. The dose of telmisartan was determined as previously described.[22,23] 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.

Every 6 months at 6, 12, or 18 months on age, the physical parameters and serum levels of triglyceride (TG), total cholesterol (T-cho), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (LDL-cho) were measured by collecting 1 mL blood from internal jugular vein under a nitrous oxide/oxygen/isoflurane mixture (69/30/1%) anesthesia. The rats were then transcardially perfused with 5 μ/mL chilled heparinized saline followed by 4% paraformaldehyde in phosphate buffer under deep anesthesia with pentobarbital (20 mg/250 g rat). After decapitation, their brains were removed, and the brain weights (BrWs) were measured. All experimental procedures were approved by the Animal Committee of the Graduate School of Medicine and Dentistry, Okayama University.

Physical Parameters and Serum Substances

As physical parameters, body weight (BW) and BrW were measured at 3, 6, 12, or 18 months and 6, 12, or 18 months, respectively, by a portable balance (Sartorius Mechatronics, Goettingen, Germany). BP was also measured using a BP monitor (MK-1030, Muromachi Kikai Co, Ltd, Tokyo, Japan) at 3, 6, 12, or 18 months. Serum levels of TG, T-cho, high-density lipoprotein cholesterol, and LDL-cho were measured by a standard biochemical method in SRL, Inc (Tokyo, Japan).

Immunohistochemistry

After measuring BrW, 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 immunostainings. For immunostainings, the brain sections were pretreated with formic acid for 3 minutes, for both Aβ and pτ stainings, followed by heating 3 times with a 500 W microwave for 5 minutes in 10 mM (pH 6.0) citric acid buffer for only pτ staining. For monocyte chemotactic protein 1 (MCP-1), tumor necrosis factor alpha (TNF-α), and ionized calcium-binding adapter molecule 1 (Iba-1) immunostainings, 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 .5% periodic