# Therapeutic Effects of Pretreatment with Tocovid on Oxidative Stress in Postischemic Mice Brain

Jingwei Shang, PhD, Hongjing Yan, MA, Yang Jiao, MA, Yasuyuki Ohta, PhD, Xia Liu, MA, Xianghong Li, PhD, Ryuta Morihara, PhD, Yumiko Nakano, PhD, Yusuke Fukui, PhD, Xiaowen Shi, MA, Yong Huang, MA, Tian Feng, MA, Mami Takemoto, PhD, Kota Sato, PhD, Nozomi Hishikawa, PhD, Toru Yamashita, PhD, and Koji Abe, PhD

*Background:* Dietary supplement is an attempt to reduce the risk of ischemic stroke in high-risk population. A new mixed vitamin E-Tocovid that mainly contains tocotrienols other than tocopherol, attenuated the progression of white matter lesions by oral in humans. However, the effect of Tocovid on ischemic stroke has not been examined. In the present study, we assessed the therapeutic effects of Tocovid pretreatment on transient middle cerebral artery occlusion (tMCAO) in mice. *Materials and Methods:* After pretreatment with Tocovid (200 mg/kg/d) or vehicle for 1 month, 60-minute tMCAO was performed, and these mice were examined at 1 day, 3 days, and 7 days after reperfusion. We histologically assessed the effects of Tocovid pretreatment on the expressive changes of oxidative stress markers, cleaved caspase-3, and LC3-II after tMCAO in mice. *Results:* We observed that Tocovid pretreatment significantly improved the rotarod time, reduced infarct volume, decreased the number of 4-HNE, nitrotyrosine, and 8-OhdG positive cells, inhibited advanced glycation end products biomarkers RAGE, CMA, and CML expressions, and increased Nrf2 and MRP1 levels with GSSG/GSH ratio decrease. Furthermore, Tocovid pretreatment greatly decreased cleaved caspase-3 and LC3-II expressions after tMCAO. *Conclusions:* The present study obviously demonstrated that Tocovid pretreatment showed neuroprotective effects against oxidative stress and at least in part by antiapoptotic/autophagic cell death in ischemic mice brain. **Key Words:** Apoptosis—autophagic cell death—oxidative stress—Tocovid—ischemic stroke.


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

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Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Jingwei Shang, and Koji Abe. Acquisition of data: Jingwei Shang, Hongjing Yan, Yang Jiao, Yasuyuki Ohta, Xia Liu, Xianghong Li, Ryuta Morihara, Yumiko Nakano, Yusuke Fukui, Xiaowen Shi, Yong Huang, Tian Feng, Mami Takemoto, Kota Sato, Nozomi Hishikawa, and Toru Yamashita. Analysis and interpretation of data: Jingwei Shang, Hongjing Yan, Yasuyuki Ohta, Yumiko Nakano, and Ryuta Morihara. Drafting of the manuscript: Jingwei Shang and Koji Abe. Critical revision of the manuscript for important intellectual content: Jingwei Shang and Koji Abe. Statistical analysis: Jingwei Shang, Nozomi Hishikawa, and Yasuyuki Ohta. Study supervision: Koji Abe.

Address correspondence to Koji Abe, PhD, Department of Neurology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. E-mail: p4wi2ykv@yahoo.co.jp.

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## Introduction

Ischemic stroke (IS) is a major cause of death and disability worldwide. Due to major limitations and a narrow 3- to 4.5-hour treatment window of thrombolysis,[1] only a small percentage of patients with acute IS receive timely and effective therapy. Dietary supplements could be alternative help to prevent IS. Vitamin E is a potent antioxidant that protects organisms against reactive oxygen species and reactive nitrogen species mediated damage, and plays an essential role in neuronal maintenance and survival of the cultured cortical neurons.[2,3] Moreover, vitamin E supplementation provides beneficial effects on neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.[4,5]

Natural vitamin E is the term for a group of tocopherols and tocotrienols, each with the 4 α, β, γ, and δ analogs, determined by the number and position of methyl groups on the chromanol ring.[6] Since the discovery of vitamin E in 1922, vitamin E regulation of disease has been extensively studied in humans, animal models, and cell systems, mainly focusing on α-tocopherol. In contrast, only 1% of research has been conducted on tocotrienols until 2012.[7] Recent studies suggested that tocotrienols more evenly distribute than tocopherol in the phospholipid bilayer and have more effective interaction with lipid peroxyl radicals in membrane environments,[8] possess neuroprotective properties at concentrations within the physiological relevant range,[9] and exhibit anti-inflammatory, anticancer, and cholesterol-lowering properties.[7,10,11]

In contrast, some previous studies also reported that vitamin E has nonantioxidant function,[12,13] and antioxidant vitamins as food supplements have no beneficial effects in the primary prevention of stroke.[14] But a recent clinical trial reported that a new mixed vitamin E-Tocovid attenuated the progression of white matter lesions by oral in humans.[9] However, the effect of Tocovid on IS has not been examined. Therefore, in the present study, we aimed to investigate the effects of pretreatment with this new mixed vitamin E-Tocovid on the oxidative stress, apoptosis, and autophagic cell death in a mouse model of acute IS.

## Materials and Methods

### *Experimental Groups and Drug Treatment*

Male ICR mice (6 weeks old, body weight 23-25 g; SLC, Shizuoka, Japan) were newly accommodated in standard mouse cages under conventional laboratory conditions with a 12/12 hours light-dark cycle and constant room temperature at around 23°C and humidity for 1 month. All animal experiments were performed in compliance with a protocol approved by the Animal Committee of the Graduate School of Medicine and Dentistry, Okayama University (OKU-2016-515).

The above mice were randomly divided into 3 groups: sham group (.5% carboxymethyl cellulose sodium salt; n = 17), vehicle group (.5% carboxymethyl cellulose sodium salt; n = 51), and Tocovid (α-tocotrienol 12.4%, β-tocotrienol 2.5%, γ-tocotrienol 19.2%, δ-tocotrienol 6.3% and α-tocopherol 11.3%) pretreated group (200 mg/kg per day; n = 51) orally once a day for 1 month.

### *Focal Cerebral Ischemia*

After 1 month of pretreatment, the mice of the vehicle and Tocovid pretreated groups were anesthetized with a mixture of nitrous oxide/oxygen/isoflurane (69%:30%:1%) during surgical preparation with an inhalation mask. Body temperature was monitored and maintained at 37 ± .3°C by using a heating pad during the surgical procedure. The right middle cerebral artery was occluded by inserting an 8-0 nylon filament thread with silicon coating through the right common carotid artery according to our previous reports.[15,16] After 60 minutes of transient middle cerebral artery occlusion (tMCAO), the filament was gently removed to restore blood flow in the middle cerebral artery territory. To confirm the effects of tMCAO, regional cerebral blood flow of the right frontoparietal cortex region was measured under general anesthesia before, during tMCAO, and at reperfusion through a laser Doppler flowmeter probe (model ALF21; Advance, Tokyo, Japan) that was attached to the surface of the ipsilateral cortex to monitor regional cerebral blood flow.

### *Neurobehavioral Tests*

We assessed the Bederson score,[17] rotarod time (MK 132 670; Muromachi Kikai Co., Tokyo, Japan)[18,19] and corner test[20] in a blinded fashion before tMCAO and on 1, 3, and 7 days after tMCAO.

### *Tissue Preparation*

For histological examinations, the mice (n = 6) were deeply anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg) and transcardially perfused with ice-cold phosphate-buffered saline (PBS), and then ice-cold 4% paraformaldehyde (PFA) in .1 mol/L phosphate buffer. The whole brain was removed and immersed in the same fixation for 12 hours at 4°C. After washing with PBS, the tissues were transferred into 10%, 20%, and 30% (wt/vol) sucrose gradients and then embedded in powdered dry ice and stored at –80°C. Twenty micrometer-thick sections were prepared using a cryostat at –18°C and mounted on silane-coated glass slides.

### *Infarct Volume Measurement*

For quantitative analysis of infarct volume, the sections were stained with hematoxylin and eosin and observed with a light microscope (Olympus BX-51; Olympus Optical, Tokyo, Japan). The area of the infarct