

Neuroprotective Effects of Tocovid Pretreatment in a Mouse Stroke Model

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Background: Tocovid is a new combination of tocotrienols and tocopherol, both of which are neuroprotective agents for preventing cerebral infarction in mice. However, the effects of tocovid on anti-inflammation in ischemic model remain elusive. In the present study, we assessed the effects of Tocovid pretreatment on anti-inflammatory effects after transient middle cerebral occlusion (tMCAO) in mice. *Materials and Methods:* We evaluated the therapeutic and anti-inflammatory effects of tocovid pretreatment (200 mg/kg per day, for 1 month) on mice brain under 60 minutes of tMCAO. The expressive changes of inflammatory markers were observed after tMCAO in mice. *Results:* Tocovid pretreatment greatly improved the mice neurobehaviors, reduced infarct volumes and decreased expressions of inflammatory markers such as tumor necrosis factor- α (TNF- α), monocyte chemoattractant protein-1 (MCP-1) and ionized calcium binding adapter molecule-1 (Iba-1), and improved the damage of neurovascular units including matrix metalloproteinase 9, IgG and collagen IV after tMCAO. *Conclusions:* Our present findings demonstrated that oral tocovid pretreatment showed obviously neuroprotective and at least in part by anti-inflammatory effects in ischemic mice brain. **Key Words:** Neuroinflammation—neurovascular unit dissociation—tocovid—transient middle cerebral occlusion.

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Introduction

Ischemic stroke is the major cause of disability and death in the world, and causes acute remarkable inflammation in brain.¹ Activated macrophages and microglia induce

inflammation in the ischemic brain,² and the amelioration for activated macrophage and microglia is essential for neuroprotection.³ Combined with the neuroprotection therapy, protective therapy for neurovascular unit (NVU) including astrocytes and endothelial cells dramatically improved brain damages after cerebral infarction.⁴

Recent studies demonstrated the protective effects of dietary supplements such as Twendee X and olive oil for ischemic brain.^{5,6} Vitamin E reacts with oxygen radicals and prevents neuronal apoptosis caused by cerebral ischemia and reperfusion.⁷ A natural mixture of vitamin E-Tocovid is a combination of tocotrienols and tocopherol, both of which are effective agents for preventing cerebral infarction in mice.⁸ In the present study, we focused on the neuroprotective and anti-inflammatory effects of tocovid on the mice brain under transient middle cerebral occlusion (tMCAO).

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Materials and Methods

Animals and Drug Presentation

Male ICR mice aged 6 weeks (23-25g body weight) were provided from Disease Model Cooperative Research Association (Kyoto, Japan). The mice were maintained under conditions with a 12 hours light-dark cycle, and gave a normal diet. Mice were randomly divided into vehicle or tocovid pretreatment group. The tocovid group received tocovid (including α -tocopherol 11.3%, α -tocotrienol 12.4%, β -tocotrienol 2.5%, γ -tocotrienol 19.2%, and δ -tocotrienol 6.3%; 200 mg/kg per day; n = 51) orally for 1 month, vehicle group (n = 51) received the equal amounts of saline until 24 hours before tMCAO operation and sham group (n = 17). The right middle cerebral artery was occluded by inserting a 6-0 surgical nylon thread with silicon coating through the common carotid artery according to our previous reports.^{9,10} After 60 minutes of tMCAO, the nylon thread was gently removed to reperfuse the middle cerebral artery. Mice were sacrificed on 1, 3, or 7 days after the tMCAO. All experimental procedures were approved by the Animal Committee of the Graduate School of Medicine and Dentistry, Okayama University (OKU-2016-515).

Neurobehavioral Tests

The neurological functions were assessed using Bederson score, rotarod time and corner test in a blinded fashion. Neurological deficits were measured using the Bederson score before tMCAO and on 1, 3, and 7 days after tMCAO: grade 0, normal; grade 1, forelimb flexion; grade 2, decreased resistance to lateral push and forelimb flexion but without circling; grade 3, with circling.¹¹ An accelerating rotarod machine was used to estimate the motor performance at the same time point with Bederson score. Recorded the score and all the data for analysis.^{12,13} For corner test, 2 card board pieces were made at an angle of 30° to shape into a corner, and then mouse entered into the corner. The mouse turns either left or right, but the ischemic mouse preferentially turns toward the nonimpaired side. After this test was repeated 10 times in each mouse, the number of right turns was recorded.¹⁴

Nissl Staining

After transcardially perfused with chilled phosphate-buffered saline (PBS, pH 7.4), brains were fixed in 4% paraformaldehyde for 24 hours at 4°C (n = 8 per group). Brains were then incubated in 20% sucrose for at least 24 hours at 4°C and instantly frozen in liquid nitrogen. Twenty-micrometer-thick frozen coronal sections were cut on a cryostat at -20°C and mounted on silane-coated glass slides. For quantification of infarct volume, the brain sections were stained with cresyl violet as Nissl staining and examined by microscopy (BX-51;

Olympus Optical, Tokyo, Japan). The cerebral infarct area was measured by using the Image J software (National Institute of Health), and the volume was calculated as our previous report.¹⁵

Single Immunohistochemical Analysis

The brain sections (20 μ m) were incubated with .3% H₂O₂/ methanol for 30 minutes to block endogenous peroxidase activity and then with 5% bovine serum albumin in 50 mM PBS, pH 7.4, containing .1% triton at room temperature for 1 hour. Slides were incubated with one of

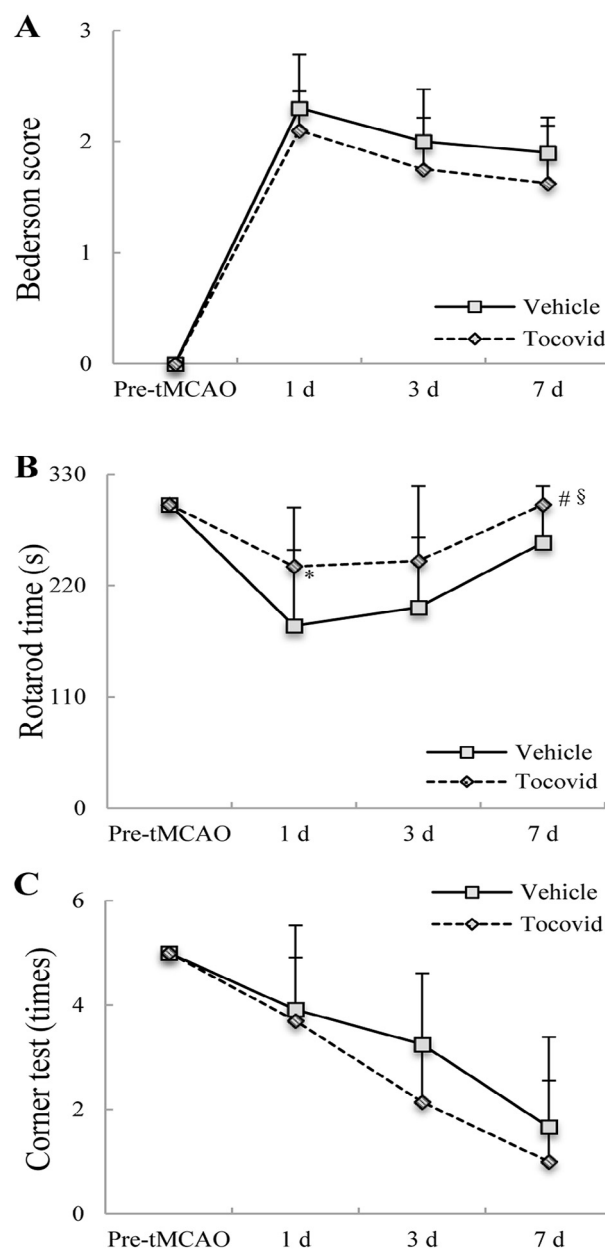


Figure 1. Clinical scores of Bederson score (A), rotarod time (s) (B), and times of corner test (C) before transient middle cerebral artery occlusion (tMCAO) and on 1, 3, and 7 days after tMCAO. * $P < .05$ versus vehicle group; # $P < .05$ versus 1 day; § $P < .05$ versus 3 days.

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