4′-O-β-D-Glucosyl-5-O-Methylvisamminol, A Natural Histone H3 Phosphorylation Epigenetic Suppressor, Exerts a Neuroprotective Effect Through PI3K/Akt Signaling Pathway on Focal Cerebral Ischemia in Rats

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■ BACKGROUND: **A bursting inflammation has been observed that compromises neurologic function in patients who experience stroke. We sought to examine the neuroprotective efficacy of 4′-O-β-D-glucosyl-5-O-methylvisamminol (OGOMV), a novel histone H3 phosphorylation epigenetic suppressor) in a transient middle cerebral artery occlusion (tMCAO).**

■ METHODS: **A rodent tMCAO model was used. Administration with 400 μg/kg/day OGOMV was initiated 12 hours before (prevention) and 1 hour after animals were subjected to tMCAO (reversal). The cerebral cortex was harvested to examine protein kinase B (PI3D/Akt), 5-bromo-2′-deoxyuridine (Western blot), and caspases (reverse-transcription polymerase chain reaction). In addition, cerebrospinal fluid samples were collected to examine interleukin 1-β, interleukin-6, monocyte chemoattractant protein-1, and tumor necrosis factor-α (reverse-transcription polymerase chain reaction).**

■ RESULTS: **Cortical 5-bromo-2′-deoxyuridine and phospho-PI3D/Akt were reduced in tMCAO animals, compared with the healthy controls but increased in the OGOMV treatment and prevention groups. Activated cortical caspase-3,-6, and -9a as well as increased IL-1β and TNF-α levels were observed in the tMCAO animals ($P < 0.05$). Both prevention and treatment with OGOMV significantly reduced cleaved caspase-3 and -9a groups, but no significant change in caspase-6 was noted. Perifosine, an Akt inhibitor, was added to reduce the bioexpression of phospho-P13D/Akt, and Bcl-2 level and increased cleaved caspase-9a level in both OGOMV prevention and treatment tMCAO groups ($P > 0.05$).**

■ CONCLUSION: **Our study suggests that OGOMV could exert a neuroprotective effect by inhibiting the P13D/Akt protein, attenuating inflammation, and cleaved caspase-3– and -9a–related apoptosis. This study also lends credence to support the notion that the prevention of OGOMV could attenuate proinflammatory cytokine mRNA and late-onset caspases in tMCAO and merits further study.**

INTRODUCTION

Globally, stroke remains the third major cause of death and disability in the population older than 60 years of age. As estimated by the World Health Organization, more than 15 million people worldwide suffer a stroke per year.[1,2]

Key words
- 4′-O-β-D-glucosyl-5-O-methylvisamminol
- Phosphatidylinositol-3-kinase
- Protein kinase B
- Transient middle cerebral artery occlusion
- Tumor necrotic factor-α

Abbreviations and Acronyms
Bad: Bcl-2–associated death promoter
BrdU: 5-Bromo-2′-deoxyuridine
BWI: Brain water index
CSF: Cerebrospinal fluid
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
IL-1β: Interleukin-1β
IL-6: Interleukin-6
iNOS: Inducible nitric oxide synthase
LDS: Lithium dodecyl sulfate
MCP-1: Monocyte chemoattractant protein-1
MDI: Motor deficit index
MLPT: Modified limb-placement test
MVNS: Modified Voetsch neuro-scores
PBS: Phosphate-buffered saline
PI3K: Phosphatidylinositol 3,4,5 triphosphates kinase
ROS: Reactive oxygen species
rt-PCR: Reverse transcription polymerase chain reaction
TNF-α: Tumor necrotic factor-α

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Citation: World Neurosurg. (2016) 89:474-488.
http://dx.doi.org/10.1016/j.wneu.2016.01.061

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

Among them, nearly 6 million die and 5 million are left permanently disabled. Ischemic strokes, a major subcategory caused by an interruption of focal cerebral blood flow, have become a point of interest. As diagnostic technologies have advanced, therapies aimed at reversing this condition have partly improved but mostly have disappointed.[3,4] An increased body of evidence points to 2 hypotheses: the role of oxidative stress and free oxide radicals subsequent to the primary stopping of blood supply[5-10] and the role of inflammation in the development and maintenance of ischemia–reperfusion injury.[11-14] In Mazzotta et al.'s study,[15] proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) aggravated the severity of reperfusion damage in stroke patients.

Leukocyte transmigration and activated endogenous microglia subsequent to a decayed tight junction of blood–brain barrier (BBB) have been shown to play a role in aggravating stroke-induced ischemia–reperfusion injury.[5,6,16] In the case of cerebral infarction, oxidative stress may increase oxygen free radicals and elicit a cascade of inflammation, which promotes activated inflammatory cytokines.[12,13,17] Proinflammatory cytokines are neurotoxic and hasten neuronal apoptosis, which corresponds to the findings of Loddick and Rothwell, where rhIL-1ra, a recombinant human interleukin-1 receptor antagonist, is able to protect endogenous interleukin-1–induced neuron damage.[18] In addition, torn endothelia and neutrophil transmigration and attraction take part in the breakdown of the integrity of BBB during stroke. A reasonable hypothesis is that preventing the onset of the robust inflammatory cascade may be helpful in limiting brain damage.

Protein kinase B, known as Akt, has been observed in phosphatidylinositol 3,4,5 triphosphates kinase (PI3K)-kinase/Akt/nitric oxide synthase (NOS) signaling, endothelial cell migration, and angiogenesis in studies of ventilator-induced pulmonary injury.[19-23,48] In the observation of oxygen deprivation–induced cerebral ischemia, Kawasaki et al.[24] showed activation of PI3K/Akt, the mammalian target of the rapamycin pathway, is decisive in neuron proliferation and apoptosis. Through activated tyrosine kinases, integrins, cytokine receptors, and G-protein-coupled receptors, the Akt cascade is onset to promote the activation of phosphatidylinositol 3,4,5 trisphosphate by PI3K, which negatively adjusts thrombogenicity, vascular permeability, inflammation, and neuron protection–related genes.[20] In Kilic et al.'s study,[25] statins are able to promote the activating phosphorylation of Akt at serine 473 by inducing Akt translocation to the plasma membrane of endothelial cells. By activating the PI3K/Akt pathway, it significantly reduces infarct size in cardiac ischemia.

4′-O-β-D-glucosyl-5-O-methylvisamminol (OGOMV), extracted from *Saposhnikovia divaricata*, has been used in the treatment of cardiovascular accidents, inflammation, and cancer in the Orientals.[26-31] OGOMV is able to abrogate the mitotic cell-cycle progression and proinflammatory gene expression through inhibition histone H3 phosphorylation in a HeLa S-3 cell culture.[32] Accumulated evidence demonstrates histone phosphorylation at Ser10 is critical in the progression of cellular mitosis and provokes proinflammatory cytokines during the early stage of interphase.[33] Like other histone deacetylase inhibitors that target the regulation of histone proteins and the acetylation of α-tubulin and exerting neuroprotection in cerebral vascular accident and traumatic brain injury, recent studies have focused on the subcategory related to neuroinflammation, such as sporadic amyotrophic lateral sclerosis, Alzheimer disease, and Parkinson disease.[34,35]

Given the talented effect of OGOMV on the opposing stimulation of proinflammatory cytokines and its potent effects on cortical dysfunction, the rat transient middle cerebral artery occlusion (tMCAO) model was used to test OGOMV, a natural histone-3 deacetylase inhibitor, on tMCAO-induced ischemia–reperfusion injury and associated cerebral apoptosis.

MATERIALS AND METHODS

OGOMV ($C_{22}H_{28}O_{10}$, molecular weight = 452), characterized as a natural, potent histone H3 phosphorylation inhibitor, was purchased from Baoji Plant Bio-Engineering Co., Ltd., Shaanxi, PRC. Monoclonal anti-rat interleukin-1β (IL-1β), IL-6, monocyte chemoattractant protein-1 (MCP-1), and TNF-α antibodies were obtained from Abcam (Cambridge, Massachusetts, USA), BD Transduction Lab (BD Biosciences, San Jose, California, USA), Upstate Biotech (Lake Placid, New York, USA), and Santa Cruz Biotechnology, Inc. (Santa Cruz, California, USA). Polyclonal anti-rat Anti-PI3K antibody (PI3K [P85] antibody), anti-phospho-PI3K antibody (phospho-PI3K [P85] [Tyr458]/[p55] [Tyr199] antibody), anti-Akt antibody (Akt antibody), and anti-phospho-Akt antibody (phospho-Akt (Ser473)) were obtained from Cell Signaling Technology (Beverly, Massachusetts, USA). Horseradish peroxidase–conjugated goat anti-rabbit IgG was purchased from R&D Systems, Inc (Minneapolis, Minnesota, USA). CNM protein extraction kits were from Biochain (Hayward, California, USA). A rabbit polyclonal NeuN, NOS, and 5-bromo-2′-deoxyuridine (BrdU) antibody was purchased from Rainbow Biotechnology (Taipei, Taiwan; distributor of Novus, Littleton, Colorado, USA). OGOMV was further purified by a high-performance liquid chromatography (LC200; Beijing Purkinje General Instrument Co., Ltd. Beijing, PRC) and prepared by S.C.W. Dimethyl sulfoxide (10 mM) worked as a solvent and vehicle.[26]

Induction of tMCAO

Fifty male Sprague–Dawley rats (350–450 g; bought from BioLasco Taiwan Co., Ltd., Taipei, Taiwan; authorized by Charles River Laboratory), were enrolled in this study. All the protocols were approved and under the supervision of the University of Kaohsiung Medicine Animal Research Committee and in accordance with the Declaration of Helsinki (1964). The animals were anesthetized with a gas mixture of 4% halothane in oxygen (1:3) in a gas chamber. The concentration of halothane was reduced to 1% after oral–tracheal intubation; the airways were maintained via a mechanical ventilator (Harvard rodent ventilator; model 683, Harvard Instrument 10; South Natick, Massachusetts, USA). Arterial blood pressure monitoring and serial blood gas analysis were taken from a catheterized femoral artery intraoperatively.

A craniotomy with the zygomatic arch preserved was made anterior to the foramen ovale with an electric drill under a surgical microscope. A small slit in the dura was then created to identify the left middle cerebral artery. The middle cerebral artery was clipped with a Codman Slim-Line Aneurysm Clip Graft (5 × 5 mm; Codman & Shurtleff, Inc., Raynham, Massachusetts) for 30 minutes. A laser