D-Allose Attenuates Overexpression of Inflammatory Cytokines after Cerebral Ischemia/Reperfusion Injury in Gerbil

Natsuyo Shinohara, MD,* Takehiro Nakamura, MD, PhD,† Yuko Abe, MD,* Toru Hifumi, MD,* Kenya Kawakita, MD, PhD,* Aya Shinomiya, MD, PhD,‡ Takashi Tamiya, MD, PhD,‡ Masaaki Tokuda, MD, PhD,§ Richard F. Keep, PhD,‖ Tohru Yamamoto, PhD,¶ and Yasuhiro Kuroda, MD, PhD*

Background: The present study investigates the effects of D-allose, a rare sugar, on the inflammatory response after transient forebrain ischemia in the gerbil and whether it reduces oxidative stress (8-hydroxyl-2'-deoxyguanosine levels) and behavioral deficits. *Methods:* Transient forebrain ischemia was induced by occlusion of the bilateral common carotid arteries for 5 minutes. D-Allose was intraperitoneally injected immediately after ischemia (400 mg/kg). Inflammatory cytokines and oxidative damage in the hippocampus and behavioral deficits were examined 3 days after ischemia. *Results:* D-Allose administration reduced ischemia-induced cytokine production, oxidative stress, and behavioral deficits (motor and memory related). *Conclusions:* The present results suggest that D-allose reduces brain injury after transient global ischemia by suppressing inflammation as well as by inhibiting oxidative stress. **Key Words:** Ischemia—reperfusion—D-allose—inflammation—cytokines—oxidation—hyperactivity.

Introduction

Transient forebrain ischemia induces neurological deficits including learning and memory impairment. During the early period of ischemia/reperfusion injury, a transient inflammatory response is started by cytokines, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α), activated in response to ischemia.[1] That inflammatory response is thought to contribute to brain injury after global ischemia.[2]

Rare sugars, such as D-allose, are monosaccharides that exist only rarely in nature. The biological effects of rare sugars remain largely unknown. Hossain et al[3,4] found that D-allose protects the liver during transplantation and during ischemia/reperfusion injury. In kidney, Ueki et al[5] found that D-allose reduces ischemia/reperfusion injury in rats in part by inhibiting inflammation. That group also found that D-allose inhibited lipopolysaccharide-induced increases in serum and renal TNF-α, renal cytokine-induced neutrophil chemoattractant-1 and myeloperoxidase concentrations, as well as the subsequent neutrophil-mediated renal injury.[6]

In global cerebral ischemia, D-allose treatment started before ischemia protects against hippocampal cell death and behavioral deficits.[7] In focal cerebral ischemia, D-allose treatment started during ischemia protects against brain injury and neurological deficits and also suppresses inflammation, as

From the *Emergency Medical Center, Kagawa University Hospital, Miki, Japan; †Department of Medical Technology, Kagawa Prefectural University of Health Sciences, Takamatsu, Japan; ‡Department of Neurological Surgery, Kagawa University Faculty of Medicine, Miki, Japan; §Department of Cell Physiology, Kagawa University Faculty of Medicine, Miki, Japan; ‖Department of Neurosurgery, University of Michigan, Ann Arbor, MI, USA; and ¶Department of Molecular Neurobiology, Kagawa University Faculty of Medicine, Miki, Japan.

Received November 3, 2015; revision received December 19, 2015; accepted January 20, 2016.

This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

Address correspondence to Takehiro Nakamura, MD, PhD, Department of Medical Technology, Kagawa Prefectural University of Health Sciences, 281-1 Hara, Mure, Takamatsu, Kagawa 761-0123, Japan. E-mail: tanakamu@kms.ac.jp.

1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2016.01.030

indicated by a reduced number of myeloperoxidase positive cells (e.g., neutrophils) in the brain after ischemia.[8,9] The present study investigated whether D-allose given directly after global ischemia in the gerbil would protect against brain injury and suppress ischemia-induced overexpression of inflammatory cytokines as a potential mechanism underlying reduced inflammation.

Materials and Methods

Animals

Animal protocols were approved by the Animal Committee of Kagawa University Faculty of Medicine. Male Mongolian gerbils (SLC, Hamamatsu, Japan), with a body weight of 60-80g, were used for all experiments. Food and water were available ad libitum.

Induction of Ischemia

Transient global ischemia was induced by a 5-minute occlusion of bilateral common carotid arteries using microaneurysm clips (Sugita Clip; Mizuho, Nagoya, Japan) under sodium pentobarbital (30 mg/kg i.p.) anesthesia. Rectal temperature was maintained at 36.5°-37.5° using a feedback-controlled heating pad (CMA, Stockholm, Sweden). After recirculation, the temperature was maintained at 37° for 60 minutes. Sham-operated control animals received the same operation except for the carotid artery occlusion. Blood glucose (samples from tail vein) and blood pressure (tail cuff method, BP-98A; Softron, Tokyo, Japan) were measured before and 3 hours after ischemia as physiological parameters (n = 6).

Experimental Groups

In the first set of experiments, the effect of D-allose (98% purity; Rare Sugar Research Center, Kagawa University, Kagawa, Japan) on ischemia-induced functional deficits and spontaneous alternation deficits was examined 3 days after cerebral ischemia/reperfusion injury. Thirty gerbils were divided randomly into sham, ischemia (vehicle : saline), and D-allose treatment at 200 mg/kg immediately after ischemia and 400 mg/kg immediately and 3 hours after ischemia (n = 6 per group). D-Allose was injected intraperitoneally after ischemia. The second set of experiments examined the effect of D-allose on ischemia-induced expression of inflammatory cytokines. There were 3 groups of animals: sham, ischemia, and 400 mg/kg D-allose treatment (n = 3 per group). In the third set, the effect of D-allose on oxidative stress was examined 3 days after a sham operation or cerebral ischemia with and without D-allose treatment (n = 3 per group).

Assessment for Motor Functional Deficits

Locomotor activity was used to assess functional outcome. The gerbils were placed in a transparent cage and activity was assessed every hour over a period of 24 hours 3 days after brain ischemia using photobeam interruption sensors (LOCOMO LS-8; Melquest, Toyama, Japan). The number of beam breaks was evaluated as locomotor activity.[8]

Assessment for Spontaneous Alternation Deficits

Y-maze spontaneous alternation is a behavioral test for measuring the willingness of rodents to explore new environments. Y-maze was used to assess spontaneous alternation deficits 3 days after brain ischemia. The number of arm entries and the number of triads were recorded to calculate the alternation percentage.

Enzyme Immunoassay for Cytokines

The gerbils were reanesthetized and decapitated 3 days after ischemia/reperfusion. Brains were removed and the bilateral hippocampi dissected and homogenized. The concentrations of the inflammatory cytokines, TNF-α, IL-1β, and IL-6, were determined by ELISA kit (Thermo Scientific, Waltham, MA, USA). Optical density was measured at 450 nm. The data, expressed as picogram per milliliter, were calculated on the basis of linear calibration curves generated with TNF-α, IL-1β, and IL-6 standard solutions.

Enzyme Immunoassay for DNA Oxidation

DNA oxidative damage was examined by 8-hydroxyl-2′-deoxyguanosine (8-OHdG) assay. Hippocampi were homogenized for DNA extraction (Dojindo Molecular Technologies, Kumamoto, Japan). 8-OHdG levels were determined by ELISA kit (Japan Institute for Control of Aging, Shizuoka, Japan). Optical density was measured at 450 nm. The data, expressed as picogram per microgram of DNA, were calculated on the basis of linear calibration curves generated with 8-OHdG standard solutions.[10]

Statistics

All data are express as mean ± standard deviation. The significance of differences was assessed with a 1-way ANOVA followed by Turkey's post hoc test. *P* values less than .05 were considered statistically significant.

Results

Physiological Parameters

All physiological parameters were measured immediately before and 30 minutes after ischemia and D-allose treatment (200 and 400 mg/kg i.p. immediately after ischemia). Blood glucose and blood pressure were within the normal range (80-120 mg/dL, 80-130 mmHg, respectively). These variables were not influenced by D-allose administration (Table 1).