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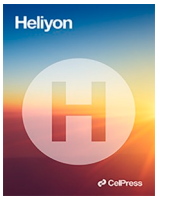

Research article

Effects of D-allose on anti-brain edema effects and reduction of tumor necrosis factor-alpha and interleukin-6 in the water intoxication model

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ABSTRACT

Background: Rare sugars, which exist only in very small quantities in nature, have recently attracted attention for their various biological functions in medicine. Among them, D-allose is known to have cytoprotective effects by antioxidant effects. In this study, we investigated whether the antioxidant effects of D-allose reduce brain edema in a water intoxication model of cytotoxic brain edema. Methods: Mice were injected intraperitoneally with distilled water (10 % of body weight) to create a model of brain edema. D-allose was administered orally at 400 mg/kg 30 min before the model was created. Two hours later, the degree of brain edema was measured by the dry-weight method to determine whether D-allose reduced brain edema. As an index of antioxidant effects, we measured changes over time in inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6) induced by the water intoxication model, and whether D-allose reduced inflammatory cytokines 4 h after model creation. Results: Administration of D-allose significantly suppressed brain edema formation of the water-intoxication model. And it significantly reduced inflammatory cytokines (tumor necrosis factor-alpha, interleukin-6). These results suggest that the antioxidant effect of D-allose exerts an anti-inflammatory effect and reduces brain edema.

1. Introduction

Brain edema is a condition that is caused by a brain injury due to cerebral ischemia, cerebral hemorrhage, trauma, or other causes. These results in abnormal intracellular or extracellular accumulation of fluid, or sometimes both, in the brain parenchyma. The worsening of brain edema increases intracranial pressure, causing brain herniation and other problems that adversely affect the neurological prognosis [1]. However, no direct treatment for brain edema has been established and is limited to symptomatic treatment and treatment of the primary disease. There is an urgent need to establish a treatment for brain edema itself to avoid serious damage.

Abbreviations: TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6.

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Rare sugars are sugars that are rare in nature and have attracted attention for their various biological functions in medicine [2]. Recently, D-allose, a rare sugar, has been suggested to have antioxidant properties in various animal models. This antioxidant effect of D-allose has been reported to be anti-inflammatory, BBB protective, and to reduce edema caused by cerebral infarction [3–8]. Currently, brain edema is widely understood to be broadly classified as cytotoxic or vasogenic brain edema, depending on whether the BBB is disrupted or not, as proposed by Klatzo et al. [9]. However, it is difficult to clearly distinguish between them in terms of actual pathology, and the types of brain edema also include a mix of the two. We previously reported that D-allose shows antioxidant effects in a cerebral ischemia model of vasogenic brain edema, improving behavior and reducing DNA damage [10]. Therefore, this study investigated whether D-allose exerts anti-edema effects on a model of brain edema caused by water intoxication. Water intoxication models are generally classified as cytotoxic brain edema models. But it is believed that brain damage from cytotoxic brain edema increases the permeability of the BBB and that brain edema gradually transitions to vasogenic brain edema [11]. We will examine whether the antioxidant effect of D-allose, which was effective in the vasogenic brain edema model, is useful in reducing edema in the water intoxication model.

The presence of inflammatory cytokines is a contributing factor to the worsening of brain edema. Inflammatory cytokines are believed to influence increased BBB permeability and are released when astrocytes and other glial cells are stimulated by brain injury, thus exacerbating brain edema [12,13]. In other words, reducing the levels of inflammatory cytokines in brain edema is important because it prevents the edema from worsening. Therefore, changes in the levels of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), which are inflammatory cytokines involved in brain inflammation, were also investigated as an evaluation of the anti-brain edema effects.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (male, 18−20 w, 25−30 g, SLC Japan, Hamamatsu, Japan) were used for all experiments. Food and water were available *ad libitum*.

2.2. Induction of brain edema and measurement of brain water content

At 2 h after intraperitoneal injection of distilled water (10 % of body weight), mice from each group were anesthetized by inhalation with sevoflurane (5 %/air), immediately decapitated, and their whole brains were quickly removed and weighed to obtain the wet weight. The brains were then reweighed after heating in an oven (250 °C) for 48 h to obtain the dry weight. The percentage of water content was calculated as follows: (wet weight − dry weight)/wet weight × 100 %.

2.3. Experimental groups

In the first set of experiments, the effect of D-allose (98 % purity; International Institute of Rare Sugar Research and Education Kagawa University, Kagawa, Japan) on the water intoxication model was examined. Twelve mice were randomly assigned to the Control, Edema, and D-allose treatment (Edema-D) group (n = 4–5 per group). The Edema-D group received 400 mg/kg (40 mg/mL) of D-allose [10] dissolved in distilled water orally, while the Control and Edema groups received only distilled water orally. Brain edema was created in the Edema and Edema-D groups by rapid intraperitoneal administration of distilled water (10 % of body weight) 30 min after oral intake of distilled water only or D-allose.

The second set of experiments examined the effects of inflammatory cytokines induced by cytotoxic brain edema. In the Edema group, the whole brain was removed 2, 4, or 6 h after the creation of brain edema, TNF-α and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) methods (Thermo Scientific, Waltham, MA, USA). In the Edema-D group, brain edema was induced after oral administration of D-allose as in the first set of experiments and TNF-α and IL-6 were measured after 4 h.

2.4. Enzyme immunoassay for cytokines

The concentrations of the inflammatory cytokines TNF-α and IL-6 were determined with ELISA kits (Enzo Life Sciences, Farmingdale, NY, USA). The optical density was measured at 450 nm. Data, expressed as pg/mL, were calculated on the basis of linear calibration curves generated with TNF-α and IL-6 standard solutions.

2.5. Statistical analysis

All data are presented as mean ± standard deviation. The significance of the differences was assessed with a one-way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test. SD values of <0.05 were considered statistically significant. All statistical analyses were performed with Bellcurve for EXCEL (Social Survey Research Information Co., Ltd.).

3. Results

3.1. Impact of D-allose on the water content of brain tissues

Mice administered orally distilled water only were defined as the Control group, and mice administered orally distilled water or 400 mg/kg D-allose and then administered an intraperitoneal injection of distilled water (10 % of body weight) were defined as the Edema (n = 5) and Edema-D (n = 4) groups, respectively. The degree of brain edema was measured using the dry-weight method. There was a significant increase in brain water content in the Edema group compared to the Control group (78.35 ± 0.40 vs. 80.54 ± 1.39; $P < 0.05$). Meanwhile, in the Edema-D group, the increase in brain water content was significantly suppressed (78.56 ± 1.05; $P < 0.05$; Fig. 1).

3.2. Time course of TNF-α and IL-6 levels in the water intoxication model

The levels of TNF-α and IL-6 were measured in the Control (no injection) and Edema groups at 2, 4, and 6 h after the creation of the water intoxication model (n = 3–4). TNF-α levels were 63.30 ± 3.94 pg/mL, 71.67 ± 14.56 pg/mL, 80.44 ± 9.73 pg/mL, and 58.96 ± 21.93 pg/mL in the Control and Edema groups at 2, 4, and 6 h, respectively (Fig. 2). IL-6 levels were 1.22 ± 0.11 pg/mL, 1.27 ± 0.33 pg/mL, 1.68 ± 0.37 pg/mL, and 1.51 ± 0.056 pg/mL in the same groups as TNF-α (Fig. 3). Both TNF-α and IL-6 reached their peaks 4 h after the creation of the model. The Control group approached the levels after 6 h (Figs. 2 and 3).

3.3. Effect of D-allose on TNF-α and IL-6 levels after inducing brain edema

As we found a tendency for hypercytokinemia in which TNF-α and IL-6 peaked 4 h after the creation of the water intoxication model, we measured the brain TNF-α and IL-6 levels of the Edema-D group 4 h after the creation of the model (Figs. 4 and 5). TNF-α and IL-6 levels increased significantly in brain tissue of the brain edema model compared to the Control group (58.83 ± 7.14 vs. 82.21 ± 7.14 pg/mL and 6.12 ± 0.48 vs. 7.80 ± 0.48 pg/mL, respectively; $P < 0.01$). Treatment with D-allose significantly suppressed the increase in TNF-α and IL-6 levels in the Edema group (64.42 ± 7.14 pg/mL and 6.50 ± 0.54 pg/mL, respectively; $P < 0.01$).

4. Discussion

Several studies have reported that D-allose has antioxidant effects [3–7]. We previously reported that D-allose showed antioxidant effects, resulting in reduced DNA damage and improved behavior in a cerebral ischemia model of vasogenic brain edema [10]. This study showed that D-allose attenuates brain edema by decreasing inflammatory cytokine levels using a model of water-intoxication brain edema created by intraperitoneal injection of distilled water.

Brain edema is classified as cytotoxic or vasogenic brain edema [9]. In general, experimental models of water intoxication are classified as models of cytotoxic edema [14]. Astrocytes stimulated by cellular brain edema increase BBB permeability by releasing inflammatory cytokines. This causes fluid to move from intravascular to extracellular spaces, and cytotoxic brain edema transitions to vasogenic brain edema with brain swelling [11,15,16]. The transition from cytotoxic edema to vasogenic brain edema due to water

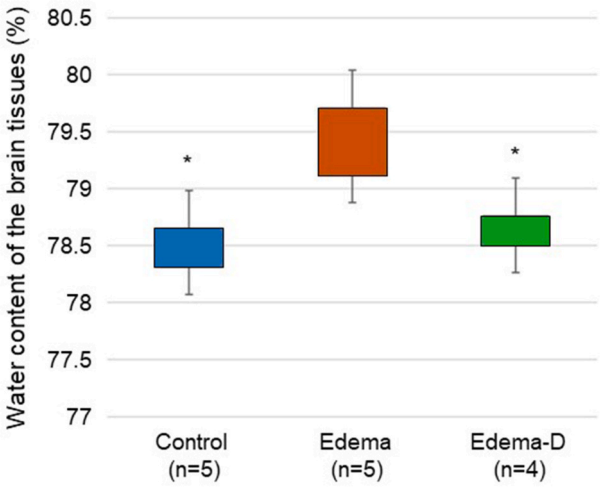

Fig. 1. Outcome of the dry-weight method for brain edema. Mice were administered an intraperitoneal injection of distilled water (10 % of body weight) after distilled water (Edema) or D-allose 400 mg/kg (Edema-D) orally. There was a significant increase in brain water content in the Edema group compared with the control group. On the other hand, in the Edema-D group, the increase in brain water content was significantly suppressed. Comparison with Edema group was statistically analyzed by one-way ANOVA (Fisher's LSD). *$P < 0.05$.

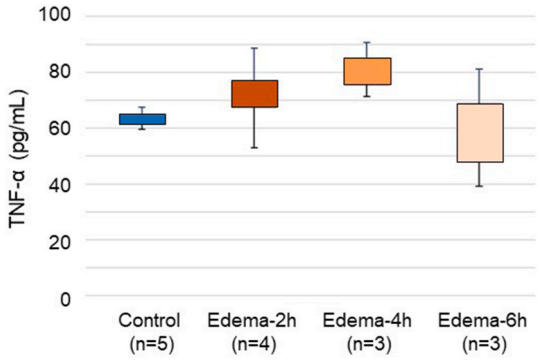

Fig. 2. Brain tissue TNF-α levels in mice administered an intraperitoneal injection of distilled water (10 % of body weight) at 2, 4, or 6 h after the creation of brain edema. TNF-α levels peaked at 4 h and tended to approach control levels after 6 h.

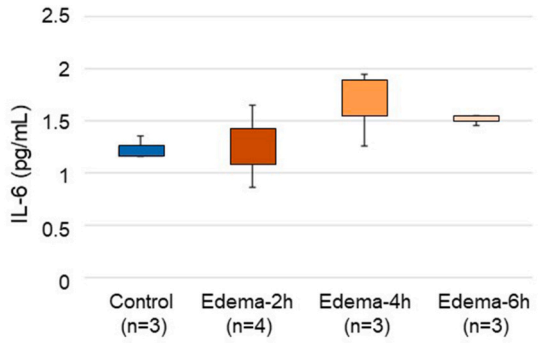

Fig. 3. Brain tissue IL-6 levels in mice administered an intraperitoneal injection of distilled water (10 % of body weight) at 2, 4, or 6 h after the creation of brain edema. IL-6 levels peaked at 4 h and tended to approach control levels after 6 h.

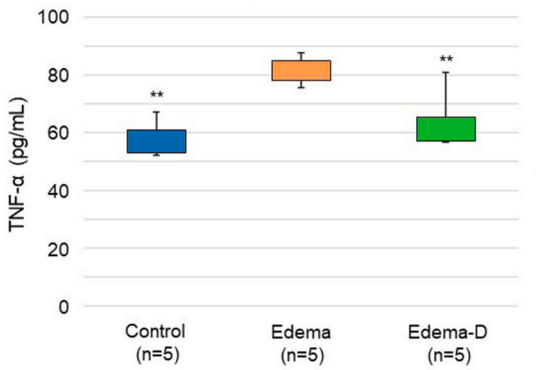

Fig. 4. TNF-α levels in brain tissue at 4 h in the Edema, Edema-D and Control group. The Edema group had significantly increased TNF-α levels compared with the Control. In the Edema-D group, the increase in TNF-α in the Edema group was significantly suppressed. Comparison with Edema group was statistically analyzed by one-way ANOVA (Fisher's LSD). **P $<$ 0.01.

intoxication begins within seconds after treatment and ends 24 h later [17,18].

In our model, brain tissue was collected 2–4 h after the creation of brain edema, which is generally classified as cytotoxic. Moreover, because it is not accompanied by other diseases, pure brain edema can be evaluated. The results showed that brain edema was significantly suppressed in the Edema-D group 2 h after the creation of the water-intoxicated brain edema model (Fig. 1), indicating that D-allose is also effective against cytotoxic brain edema. The levels of inflammatory cytokines (TNF-α and IL-6) peaked 4 h after creation in the water intoxication model (Figs. 2 and 3). D-allose already suppressed edema formation after 2 h (Fig. 1), suggesting that it has a strong anti-inflammatory function. TNF-α and IL-6 levels in the Edema-D group were significantly lower than those of the Edema group after 4 h of creation (Figs. 4 and 5), suggesting that D-allose has anti-inflammatory effects. This attenuation of brain

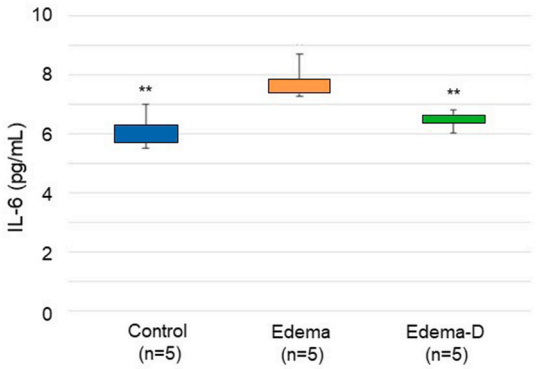

Fig. 5. IL-6 levels in brain tissue at 4 h in the Edema, Edema-D and Control group. The Edema group had significantly increased levels of IL-6 compared with the Control. In the Edema-D group, the increase in IL-6 level in the Edema group was significantly suppressed. Comparison with Edema group was statistically analyzed by one-way ANOVA (Fisher's LSD). **$P < 0.01$.

edema may be caused by osmotic diuresis; however, in the study by Shinohara et al. no increase in blood sugar was observed with 400 mg/kg of D-allose administration [10], suggesting that the decrease in brain edema in this study was purely due to the antioxidant effects of D-allose.

Although the underlying mechanisms by which D-allose ameliorates oxidative stress, neuroinflammation, and neuronal cell death are not fully understood, two mechanisms have recently been proposed for the antioxidant effects of D-allose. The first is the mechanism by which D-allose activates the peroxisome proliferator-activated receptor γ (PPARγ) pathway and exerts antioxidant effects [8]. The PPARγ pathway is also considered involved in the regulation of IL-6 [19], and the fact that D-allose attenuated IL-6 in this study may indicate that the PPARγ pathway was activated. The second mechanism is the reduction of reactive oxygen species (ROS) by mitochondria. Ishihara et al. reported that D-allose interferes with ROS production and exerts antioxidant effects [20]. ROS are also closely associated with increases in TNF-α, and by upregulating each other, they form a vicious cycle in neuroinflammation [12,13]. Since TNF-α is upstream of the PPARγ pathway [21], we hypothesize that a decrease in TNF-α may also activate the PPARγ pathway and prevent increased BBB permeability [12,13]. The fact that pre-administration of D-allose was effective in reducing brain edema in this study may be the result of a reduced BBB permeability due to a decrease in TNF-α resulting from a decrease in ROS.

In summary, the antioxidant effect of D-allose reduced inflammatory cytokines and inhibited the formation of brain edema not only in vasogenic but also in cytotoxic brain edema caused by water intoxication. The anti-brain edema effect of these rare sugars is expected to be beneficial in the establishment of a treatment for brain edema induced by various causes in clinical practice.

Ethics declarations

The study protocol was reviewed and approved by the Animal Ethics Committee of the Kagawa Prefectural University of Health and Sciences, with approval No. 3−4.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Keiichiro Irie: Writing – original draft, Investigation, Formal analysis, Data curation. **Emi Nakamura-Maruyama:** Writing – original draft. **Mai Ishikawa:** Investigation. **Takehiro Nakamura:** Writing – review & editing, Validation, Methodology, Conceptualization. **Keisuke Miyake:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Takehiro Nakamura reports article publishing charges was provided by Heliyon. Corresponding author: associate editor at Heliyon If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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