

Protection of the brain through supplementation with larch arabinogalactan in a rat model of vascular dementia

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BACKGROUND/OBJECTIVES: Vascular dementia (VaD) caused by reduced blood supply to the brain manifests as white matter lesions accompanying demyelination and glial activation. We previously showed that arabinoxylan consisting of arabinose and xylose, and arabinose itself attenuated white matter injury in a rat model of VaD. Here, we investigated whether larch arabinogalactan (LAG) consisting of arabinose and galactose could also reduce white matter injury.

MATERIALS/METHODS: We used a rat model of bilateral common carotid artery occlusion (BCCAO), in which the bilateral common carotid arteries were exposed and ligated permanently with silk sutures. The rats were fed a modified AIN-93G diet supplemented with LAG (100 mg/kg/day) for 5 days before and 4 weeks after being subjected to BCCAO. Four weeks after BCCAO, the pupillary light reflex (PLR) was measured to assess functional consequences of injury in the corpus callosum (cc). Additionally, Luxol fast blue staining and immunohistochemical staining were conducted to assess white matter injury, and astrocytic and microglial activation, respectively.

RESULTS: We showed that white matter injury in the the cc and optic tract (opt) was attenuated in rats fed diet supplemented with LAG. Functional consequences of injury reduction in the opt manifested as improved PLR. Overall, these findings indicate that LAG intake protects against white matter injury through inhibition of glial activation.

CONCLUSIONS: The results of this study support our hypothesis that cell wall polysaccharides consisting of arabinose are effective at protecting white matter injury, regardless of their origin. Moreover, LAG has the potential for development as a functional food to prevent vascular dementia.

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INTRODUCTION

Vascular dementia (VaD), which impairs cognitive abilities, is the second most common type of dementia, accounting for approximately 15-20% of all dementia patients [1,2]. VaD results from inadequate blood supply to the brain caused by occlusion or rupture of cerebral arteries [1]. Because of its diverse etiologies, VaD can be classified into various subtypes, including hypoperfusion dementia, subcortical VaD, multi-infarct dementia, and strategic infarct dementia [2,3].

Several animal models that mimic VaD have been developed to study VaD [1,4]. Of these, the bilateral common carotid artery occlusion (BCCAO) model in rats is the most commonly used. In this model, white matter lesions manifest axonal and myelin injury, vacuolization, and glial cell activation [1,4]. Thus, the rat BCCAO model mimics hypoperfusion dementia and subcortical VaD in that white matter injury is consistently observed in both subtypes [3,4]. In hypoperfusion dementia, one etiology is caused by partial or complete blockage of the carotid arteries [3,4]. In humans, carotid artery stenosis or occlusion was found

to be associated with white matter injury [3], which is observed in the regions including the corpus callosum (cc) [5,6] and optic nerve [7], a bundle of which forms the optic tract (opt) in the visual pathway (Fig. 1). In the rat BCCAO model, optic nerve injury correlates with loss of the pupillary light reflex (PLR), a reflex that controls the pupil diameter in response to light intensity [8]. Conversely, in the subcortical VaD, the most common form of VaD, one etiology is caused by partial blockage of small vessels, which also leads to white matter injury [3,9].

As there are no treatments approved by the FDA, it is imperative to develop agents to treat VaD [1,2]. We previously showed that hot water extract of ground wheat [10] and wheat bran (WBE) [11] reduced brain injury in a rat BCCAO model, demonstrating that processed wheat can be developed as a functional food for prevention of VaD. We also found that arabinoxylan, a cell wall polysaccharide consisting of arabinose and xylose, and arabinose itself were the active components responsible for the efficacy [10]. These findings suggest that any polysaccharides containing arabinose might show similar efficacies. Of the cell wall polysaccharides constituting wheat

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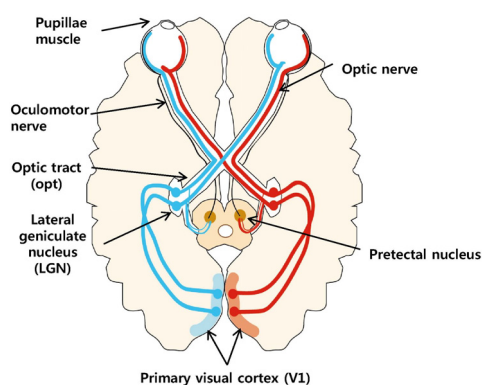


Fig. 1. A schematic diagram of the visual and pupillary light reflex (PLR) pathways. In the visual pathway, visual information reaching the retina passes through the optic nerve, optic tract (opt), and lateral geniculate nucleus (LGN) to the primary visual cortex (V1). In the PLR pathway, information for light intensity is mediated from the retina through the optic nerve, opt, pretectal nucleus, and oculomotor nerve to the pupillae muscle [22]. opt, optic tract.

cell walls, arabinogalactan-peptide is also a candidate in addition to arabinoxylan, because arabinogalactan-peptide is composed of 92% polysaccharide arabinogalactan and 8% peptides [12,13], and the arabinogalactan domain in arabinogalactan-peptide consists of arabinose and galactose [12]. To test the hypothesis that arabinogalactan-peptide in wheat is also an active component, we selected commercially available LAG as a model polysaccharide representing arabinogalactan-peptide. We then investigated whether larch arabinogalactan (LAG) supplementation could reduce white matter injury and improve PLR in a rat BCCAO model.

MATERIALS AND METHODS

Materials

LAG (82 wt % galactose and 13 wt % arabinose, along with several unidentified, minor compounds) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Eight-week-old male Sprague Dawley (SD) rats (280-300 g) were purchased from Samtaco Inc. (Osan, Gyeonggi-do, Republic of Korea). Animals were housed with diet and water available *ad libitum* before the start of the experiment, and diurnal lighting conditions and temperature-controlled environments were maintained throughout the experiment [10,11]. Experiments were conducted according to the guidelines for animal care and protocols on laboratory animal use approved by the Institutional Animal Care and Research Advisory Committee of Catholic University, Daegu, Republic of Korea (Approval No. 2013-0820-CU-AEC-10-Y).

Diet preparation

Diets containing LAG were prepared as previously described [14]. For the LAG diet (1 kg), a mixture of LAG (2 g) and corn starch (48 g) was added to 950 g of a modified AIN-93G diet [15] purchased from Unifaith Inc. (Seoul, Republic of Korea) (Table 1). For the basal diet (1 kg), 50 g of corn starch was added to 950 g of the modified AIN-93G diet.

Table 1. Composition of experimental diets

Ingredient	Modified AIN-93G diet	Basal diet	LAG diet
	(g/950 g) ¹⁾	(g/kg) ²⁾	100 mg/kg/day LAG diet (g/kg) ³⁾
Casein	250.0	250.0	250.0
Corn starch	482.5	482.5	482.5
Sucrose	100.0	100.0	100.0
Soybean oil	70.0	70.0	70.0
Mineral mix	35.0	35.0	35.0
Vitamin mix	10.0	10.0	10.0
Choline bitartrate	2.5	2.5	2.5
Corn starch	-	50.0	48.0
LAG ⁴⁾	-	-	2.0

¹⁾ A modified AIN-93G diet was purchased in a pre-mixed form.

²⁾ One kg of basal diet was prepared by adding 50 g of corn starch to 950 g of the modified AIN-93G diet.

³⁾ One kg of LAG diet was prepared by adding 50 g of a mixture of corn starch and LAG to 950 g of the modified AIN-93G diet. The LAG diet of 100 mg/kg/day refers to the corresponding dose of LAG given per kg of rat body weight per day.

⁴⁾ The amount of LAG for the LAG dose (100 mg/kg/day) was calculated based on data showing that a 300 g rat consumes 15 g of LAG diet/day. LAG, larch arabinogalactan.

Diet administration [11]

Rats were randomly divided into three groups: (1) sham (n = 6), (2) control (n = 6), and (3) LAG-treated group (100 mg/kg/day) (n = 6). The 100 mg/kg/day dose of LAG was selected based on our previous findings that WBE consisting of 2.4 wt% arabinose significantly reduced white matter injury at a dose of 400 mg/kg/day in the same rat BCCAO model [10]. Therefore, the arabinose dose that is an active component in the same rat BCCAO model [11] is equivalent to approximately 10 mg/kg/day in 400 mg/kg/day WBE. To match 10 mg/kg/day arabinose, we selected a dose of LAG at 100 mg/kg/day because LAG consists of approximately 10% arabinose. In the LAG-treated group (100 mg/kg/day), rats received LAG diet for 5 days before and 4 weeks after bilateral common carotid artery ligation. The amount of LAG diet fed to each rat at the beginning of the experiment was 15 g to give a LAG dose of 100 mg/kg/day based on the assumption that the rats weighed 300 g (Table 1). The amount of LAG diet fed to the rats then increased in proportion to the rise in rat weight because the rats gained weight as the experiment progressed. In the sham and control groups, rats received the basal diet only. Once the rats consumed all of the LAG or basal diet, more basal diet was provided *ad libitum*.

Bilateral common carotid arteries occlusion (BCCAO)

The rat bilateral common carotid arteries were ligated to mimic vascular dementia as previously described [10,11]. The male SD rats were anesthetized through isoflurane inhalation (Hana Pharmaceutical Inc., Seoul, Republic of Korea) throughout the surgical procedure. The bilateral common carotid arteries in the control and LAG-treated groups were exposed and ligated permanently with silk sutures. The rats in the sham group underwent the same experimental procedure without ligation. Four weeks after BCCAO, the PLR was examined, after which the rats were anesthetized again through isoflurane inhalation, and their brains were harvested for further study.

Pupillary light reflex (PLR) assessment

The PLR, which reflects the injury in the visual pathway, was assessed as previously described [11]. Briefly, the PLR of each rat was examined before and after the BCCAO experiments. In the PLR examination, each rat was first adapted to darkness for at least 5 min. One eye was then exposed to a beam of light from a clinical pen light (3M Korea, Ltd., Seoul, Republic of Korea). Then, after the rat was re-adapted to darkness for 1 min, the other eye was also exposed to the light. Loss of PLR in each eye was defined as failure of the pupil to constrict after exposure to a beam of light for 10 seconds.

Luxol fast blue staining

Luxol fast blue staining was performed to assess white matter injury as previously described [11]. Briefly, the harvested brain was cut into slices including the cc, opt, and internal capsule. After formalin fixation and paraffin-embedding, 5 μ m thick sections were cut from the slices. Following deparaffinization, three sections obtained from each rat brain were stained with Luxol fast blue. The severity of white matter injury was graded by Lee (the corresponding author), who was blinded to the experimental conditions performed by Lim (the first author), as normal (Grade 0), disarrangement of nerve fibers (Grade 1), formation of marked vacuoles (Grade 2), and loss of myelinated fibers (Grade 3) [16].

Immunohistochemical staining

Immunohistochemical staining was conducted to assess astrocytic and microglial activation as previously described [11]. Briefly, the sections were treated by boiling in a microwave to retrieve antigens. Next, the sections were treated with hydrogen peroxide (3%) in methyl alcohol to block endogenous peroxidases and incubated in phosphate-buffered saline containing 1% bovine serum albumin, 0.1% Triton X-100, and 5% normal goat serum to block nonspecific binding. The sections were then incubated with primary antibodies against glial fibrillary acidic protein (GFAP, 1:100, BD PharMingen, San Diego, CA, USA) or ionized calcium binding adaptor molecule 1 (Iba1, 1:200, Wako, Osaka, Japan). After washing, the sections were treated with biotinylated secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA) against GFAP and Iba1. Sections were then incubated with avidin-biotin peroxidase (Elite Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA), after which they were visualized with diaminobenzidine (Roche, Mannheim, Germany). Immunostained sections were subsequently captured using an Olympus microscope (200 \times) (Olympus Corporation, Tokyo, Japan), after which quantitative analysis was performed using the ImageJ software (NIH, v1.47). The area covered by GFAP-positive astrocytes and Iba1 positive microglia was computed as a percentage of the total area. To assess the GFAP- and Iba1-positive levels, the relative values for the control and LAG-treated groups were calculated by setting those for the sham group at 100%.

Statistical analysis

Values were expressed as the means \pm SEM. Statistical analysis for multiple comparisons consisted of one-way ANOVA followed by the Dunnett post hoc test, which were conducted using the

SPSS software (IBM SPSS Statistics; version 19, Armonk, NY, USA). For all data, Shapiro-Wilk and Levene statistics were applied a priori to verify normality and homogeneity of variances, respectively. A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

LAG Supplementation reduces white matter injury in the corpus callosum and optic tract

To examine whether LAG supplementation can reduce white matter injury, LAG (100 mg/kg/day) was supplemented for 5 days before the rats underwent BCCAO surgery, and supplementation continued for another 4 weeks after surgery (Fig. 2A). Of the regions in white matter, we focused on myelin injury incurred in the cc and opt because they are more vulnerable to hypoperfusion generated through BCCAO than other regions,

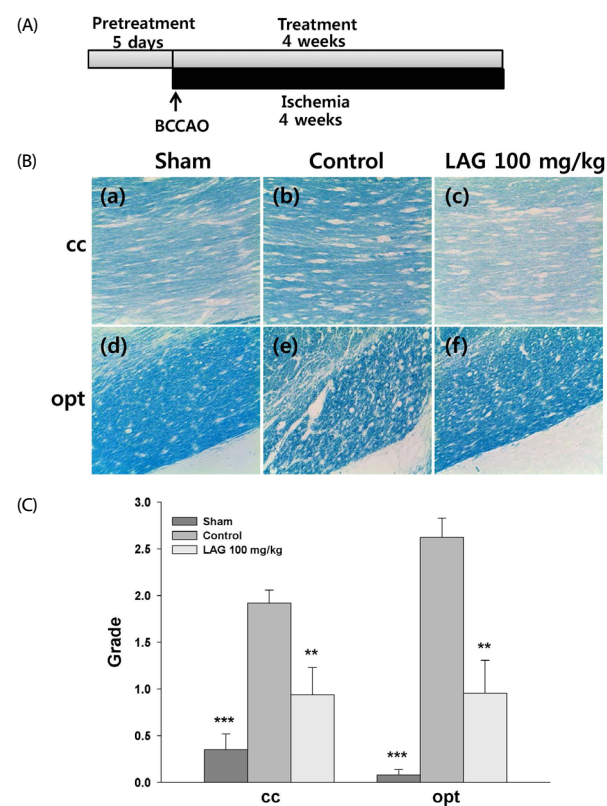


Fig. 2 Luxol fast blue staining of myelin. (A), Experimental Scheme. LAG (100 mg/kg/day) was supplemented for 5 days before the rats underwent BCCAO surgery, and supplementation continued for another 4 weeks after surgery. In BCCAO surgery, the bilateral common carotid arteries in the control and LAG-treated groups were exposed and ligated permanently with silk sutures. The rats in the sham group underwent the same experimental procedure without ligation. The rats were then anesthetized again via isoflurane inhalation, after which their brains were harvested for Luxol fast blue staining and immunohistochemical staining. (B), Representative photomicrographs of selected white matter regions stained with Luxol fast blue (200 \times): (a), (d) sham group; (b), (e) control group; (c), (f) LAG-treated group; (a)-(c) and (d)-(f) were taken from the cc and opt, respectively. (C), Quantitative analysis of white matter injury. The extent of white matter injury in the cc and opt were graded as normal (Grade 0), disarrangement of nerve fibers (Grade 1), formation of marked vacuoles (Grade 2), and loss of myelinated fibers (Grade 3). The numbers of rats used in the sham, control and LAG-treated groups were 6, 6 and 6, respectively. *** $P < 0.001$, ** $P < 0.01$ vs. control group. LAG, larch arabinogalactan; BCCAO, bilateral common carotid artery occlusion; cc, corpus callosum; opt, optic tract.

including the internal capsule, because of the levels of blood supply to the regions [11].

To assess the white matter injury that manifests disarrangement and disappearance of nerve fibers consisting of axons and the myelin sheath, Luxol fast blue staining was used to observe changes in myelin structure. In the control group, white matter injury that manifests white matter rarefaction accompanying vacuolization was observed in both the cc and opt (Fig. 2B). In contrast, in the LAG-treated group, this white matter injury was reduced in both the cc and opt, compared with the control group (Fig. 2B). The severity of white matter injury was then quantitatively assessed using a grading system (0-3) (Fig. 2C). The grading scores were significantly reduced in the LAG-treated group by 51% (0.94 ± 0.29 vs. 1.92 ± 0.14 , $P < 0.01$) in the cc, and by 63.5% (0.96 ± 0.35 vs. 2.63 ± 0.21 , $P < 0.01$) in the opt, respectively, compared with the control group (Fig. 2C). These findings indicate that LAG supplementation protects against white matter injury attributed to hypoperfusion in the rat BCCAO model.

LAG supplementation improves PLR

Because degeneration of the optic nerve that is connected to the opt causes PLR loss in a BCCAO model [8,11] (Fig. 1), we investigated whether LAG supplementation that reduced the opt injury could improve PLR. In the sham group, all six (100%) rats exhibited bilateral PLR, showing that all rats maintained normal PLR function in the sham group, as expected (Table 2). However, in the control group, five (83%) rats suffered bilateral PLR loss, while only one (17%) exhibited bilateral PLR exhibition. These findings indicate that five rats lost PLR function in both eyes, while one rat maintained normal PLR function in the control group. Conversely, two (33%) and two (33%) rats suffered bilateral and unilateral PLR loss, respectively, in the LAG-treated group, while two (33%) exhibited bilateral PLR. These findings indicate that two rats lost PLR function in both eyes, two lost PLR function in one eye, and two maintained normal PLR function in the LAG-treated group. Therefore, LAG supplementation improved PLR function by shifting two rats from bilateral PLR loss to unilateral PLR loss, and by shifting one rat from bilateral PLR loss to bilateral PLR exhibition. These findings suggest that LAG intake can improve the PLR.

LAG supplementation inhibits astrocytic activation in the cc

As chronic hypo-perfusion generated in a rat BCCAO model triggers astrocytic activation accompanying hypertrophy and

Table 2. Effect of larch arabinogalactan intake on pupillary light reflex

Experimental groups	Bilateral PLR loss ¹⁾	Unilateral PLR loss ¹⁾	Bilateral PLR exhibition ¹⁾
Sham	0 (0%) ²⁾	0 (0%)	6 (100%)
Control	5 (83%)	0 (0%)	1 (17%)
LAG-treated	2 (33%)	2 (33%)	2 (33%)

The numbers of rats used in the sham, control and LAG-treated groups were 6, 6 and 6, respectively.

¹⁾ Bilateral PLR loss, unilateral PLR loss, and bilateral PLR exhibition represent loss of PLR function in both eyes, loss of PLR function in one eye, and no loss of PLR function (normal PLR function) for each rat, respectively.

²⁾ Number and percentage indicate the number of rats in each group and 100 × (number of rats in each group/number of total rats), respectively.
PLR, pupillary light reflex; LAG, larch arabinogalactan.

proliferation of astrocytes [16,17], we investigated whether LAG supplementation can modulate astrocytic activation through immunohistochemical staining of GFAP, a biomarker specific for astrocytes in the brain [18]. In the control group, increases in the number of GFAP-positive astrocytes were observed in both the cc and opt, compared with the sham group. In contrast, in the LAG-treated group, decreases in the number of GFAP-positive astrocytes were observed in the cc compared with the sham group (Fig. 3A). The degree of astrocytic activation was then quantitatively assessed through measurement of the area covered by GFAP-positive astrocytes (Fig. 3B). The relative total area in the LAG-treated group was significantly attenuated in the cc compared with the control group (106.3 ± 5.8 vs. 148.8 ± 13.1 , $P < 0.05$), whereas the relative total area tended to be reduced in the opt compared with the control group (171.1 ± 25.3 vs. 209.9 ± 31.2 , $P > 0.05$) (Fig. 3B). These findings showed that LAG intake can inhibit astrocytic activation.

LAG supplementation inhibits microglial activation in the opt

Because chronic hypo-perfusion generated in a rat BCCAO model also triggers microglial activation accompanying morphological transformation and proliferation of microglia [16,17], we also investigated whether LAG supplementation can modulate microglial activation through immunohistochemical staining of Iba1, a biomarker specific for microglia in the brain [19]. In the control group, increases in the number of Iba1-positive microglia

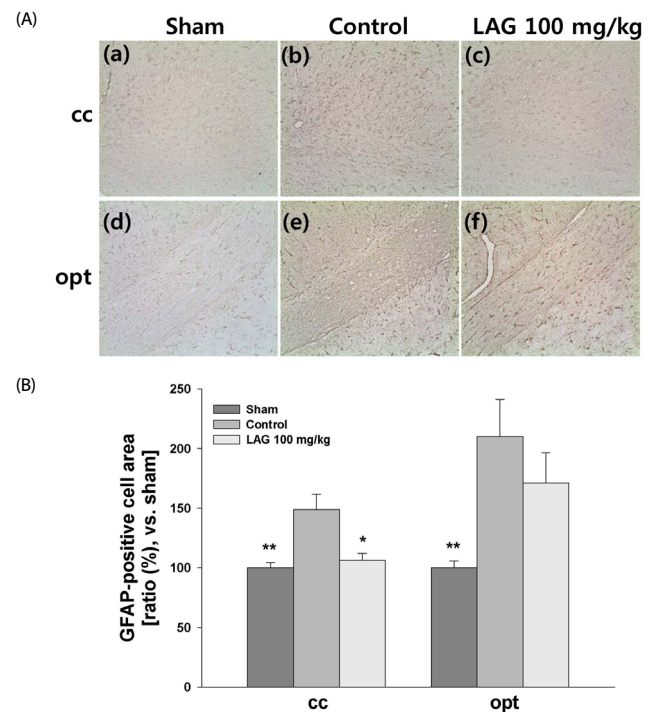


Fig. 3 Immunohistochemical staining of astrocytes. (A). Representative photomicrographs of astrocytes stained against GFAP with immunohistochemical techniques (100×): (a), (d) sham group; (b), (e) control group; (c), (f) LAG-treated group; (a)-(c) and (d)-(f) were taken from the cc and opt, respectively. (B). Quantitative analysis of GFAP-positive cells. The relative areas of GFAP-positive cells in the cc and opt were presented by setting the area of GFAP-positive cells for the sham group to 100%. The numbers of rats used in the sham, control and LAG-treated groups were 6, 6 and 6, respectively. ** $P < 0.01$, * $P < 0.05$ vs. control group. GFAP, glial fibrillary acidic protein; LAG, larch arabinogalactan.

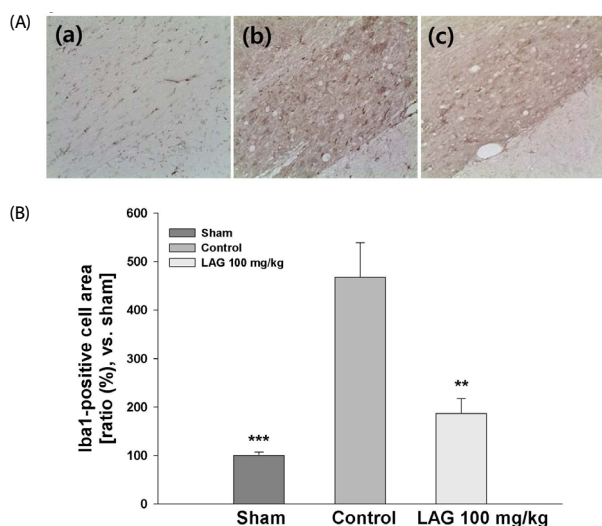


Fig. 4 Immunohistochemical staining of microglia. (A). Representative photomicrographs of microglia stained against Iba1 with immunohistochemical techniques (200 \times): (a) sham group; (b) control group; (c) LAG-treated group; (a)-(c) were taken from the opt. (B). Quantitative analysis of Iba1-positive cells. The relative numbers of Iba1-positive cells in the opt were presented by setting the number of Iba1-positive cells for the sham group at 100%. The numbers of rats used in the sham, control and LAG-treated groups were 6, 6 and 6, respectively. *** $P < 0.001$, ** $P < 0.01$ vs. control group, LAG, larch arabinogalactan; opt, optic tract.

were observed in the opt compared with the sham group. In contrast, in the LAG-treated group, decreases in the number of Iba1-positive microglia were observed in the opt compared with the sham group (Fig. 4A). The degree of microglial activation was then quantitatively assessed through measurement of the area covered by Iba1-positive microglia (Fig. 4B). The relative total area in the LAG-treated group was significantly attenuated in the opt compared with the control group (186.5 ± 31.1 vs. 468.0 ± 70.7 , $P < 0.01$) (Fig. 4B). These findings showed that LAG intake can inhibit microglial activation.

DISCUSSION

In this study, we showed that LAG supplementation reduced white matter injury in the cc and opt, bundles of myelinated axons, in a rat BCCAO model. This protection was associated with inhibition of astrocytic and microglial activation in the cc and opt, respectively. Additionally, LAG intake improved PLR. Similar observations were also made for WBE [10].

Previously, damage to the cc and opt were shown to be accompanied by activation of astrocytes and microglia in rat BCCAO models [20] and human VaD patients [21,22]. Activation of nuclear factor kappa-B (NF- κ B), an inflammatory cytokine, in astrocytes contributes to neuronal degeneration [23]. Inhibition of expression of NF- κ B by astrocytes under conditions of astrocytic activation in a mouse model of bilateral common carotid artery stenosis ameliorated myelin injury and astrocytic activation, while preserving memory function [24]. These results suggest that astrocytic activation exerts deleterious effects on myelin injury. In addition, hot water extract of ground wheat [10] and WBE [11] also reduced myelin injury and astrocytic and microglial activation, and improved visuospatial memory [10]

in a rat BCCAO model. These findings indicate that reduction of glial activation contributes to maintenance of white matter structure in VaD. Therefore, reduction of glial activation through LAG intake may also contribute to the reduced damage in the cc and opt observed in the present study.

PLR is mediated from the retina through the optic nerve, opt, pretectal nucleus, and oculomotor nerve to the pupillae muscle [25] (Fig. 1). Because LAG intake improved PLR in the model, it should protect axons involved in the PLR pathways. Therefore, injury reduction in the opt by LAG intake should help improve the PLR. In addition, it is highly likely that LAG intake also protected the retinal and optic nerves. This conclusion is supported by the findings that PLR loss was accompanied by retinal and optic nerve degeneration in rat BCCAO models [8,26]. Similar to those observed in rat BCCAO models, injury to the retinal nerve [27] and optic nerve [7] was also observed in human carotid artery occlusion, which is associated with dementia [28]. Moreover, patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a model of pure small-vessels disease that progresses to subcortical VaD, display damage to the retinal [29,30] and optic nerves [29,31]. Therefore, LAG intake may contribute to improved cognition in VaD patients through protection of the opt.

The cc is the principal interhemispheric commissure, and its integrity is crucial for cognition [32], including visual memory [33]. As expected, the cc in subcortical VaD patients shows axonal injury and astrocytic activation assessed through Luxol fast blue staining and GFAP immunohistochemical staining, respectively [34]. In addition, the cc in the subcortical VaD also shows reduced integrity when assessed through magnetic resonance imaging [35,36]. Therefore, LAG intake may contribute to improved cognition in VaD patients through protection of the cc. Taken together, the findings from this study indicate that prophylactic LAG intake can prevent VaD by protecting myelinated axons. Consistent with these findings, intake of a polysaccharides blend, including arabinogalactan as a major constituent, improved memory for healthy middle-aged adults [37].

In our previous study, we showed that supplementation of hot water extract of ground wheat, arabinoxylan, and arabinose reduced white matter injury in a rat BCCAO model [10]. In this study, we showed that supplementation of the rat diet with LAG as a model polysaccharide for arabinogalactan-peptide in wheat also reduced white matter injury in the same BCCAO model. These results suggest that arabinogalactan-peptide in wheat is also an active component. Moreover, the results support our hypothesis that any polysaccharides consisting of arabinose can also act as active components. A proposed pathway in which arabinose reduces white matter injury is shown in Fig. 5 [38]. When LAG is ingested, arabinose attached to LAG can be released from LAG through acid hydrolysis in the stomach [39]. However, not much arabinose would be released in the small intestine because LAG is not digested in the small intestine owing to a lack of endogenously secreted digestive enzymes [40]. Conversely, arabinose can also be released from LAG by microorganisms inhabiting the large intestine such as *Bacteroides thetaiotamicron*, which can cleave bonds linking the arabinose and galactan backbone by a

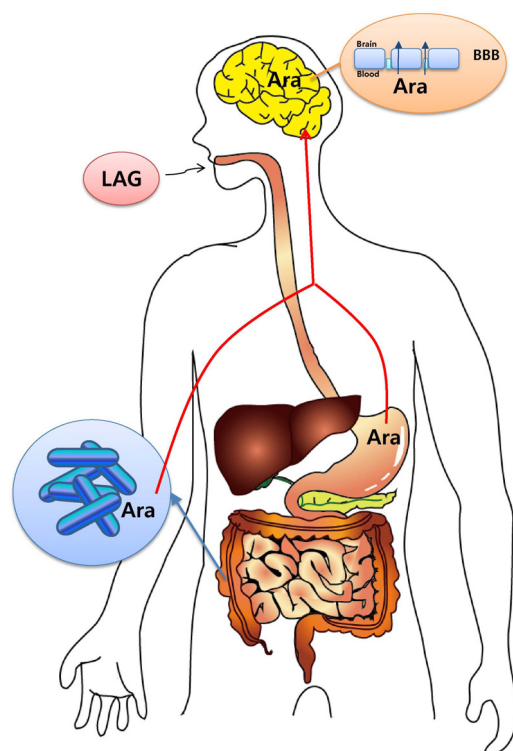


Fig. 5 A proposed pathway showing arabinose efficacy. When LAG is ingested, ara can be generated through acid hydrolysis in the stomach and through enzyme hydrolysis by microorganisms inhabiting the large intestine. The ara generated can then be absorbed into the body and penetrate across the BBB. Finally, ara in the brain reduces white matter injury in a rat BCCAO model. Modified from Ref. 16. LAG, larch arabinogalactan; ara, arabinose; BBB, blood-brain barrier; BCCAO, bilateral common carotid artery occlusion.

-arabinofuranosidase expressed by the microorganisms [38,41]. The assumption that arabinose can be released from LAG is supported by observations it was detected in the feces of mice fed a diet containing arabinogalactan [42].

LAG was approved by the United States Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS), and is currently used as a food additive to improve bowel health [43] and as an immune enhancer [43,44]. In this study, we provided further evidence of the feasibility for development of LAG as a functional food for preventing VaD, for which there are currently no treatments approved by the FDA [1,2]. Furthermore, the dose used in this study (33 days at 100 mg/kg/day for rats) was confirmed to be located in a safe range for use as a functional food based on the following findings of previous studies. Repetitive intravenous injection of LAG to rats at 500 mg/kg/day for 90 days showed no evidence of toxicity [45]. In addition, healthy human subjects ingested up to 30 g LAG for 3 weeks, which is equivalent to approximately 3 g/kg/day LAG for rats according to FDA guidelines for dose conversion between animals and human [47], and it was well tolerated [46].

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

REFERENCES

1. Venkat P, Chopp M, Chen J. Models and mechanisms of vascular dementia. *Exp Neurol* 2015;272:97-108.
2. O'Brien JT, Thomas A. Vascular dementia. *Lancet* 2015;386:1698-706.
3. Iadecola C. The pathobiology of vascular dementia. *Neuron* 2013;80:844-66.
4. Jiwa NS, Garrard P, Hainsworth AH. Experimental models of vascular dementia and vascular cognitive impairment: a systematic review. *J Neurochem* 2010;115:814-28.
5. Yamauchi H, Fukuyama H, Harada K, Nabatame H, Ogawa M, Ouchi Y, Kimura J, Konishi J. Callosal atrophy parallels decreased cortical oxygen metabolism and neuropsychological impairment in Alzheimer's disease. *Arch Neurol* 1993;50:1070-4.
6. Lin CJ, Chang FC, Chou KH, Tu PC, Lee YH, Lin CP, Wang PN, Lee IH. Intervention versus aggressive medical therapy for cognition in severe asymptomatic carotid stenosis. *AJNR Am J Neuroradiol* 2016;37:1889-97.
7. Terelak-Borys B, Skonieczna K, Grabska-Liberek I. Ocular ischemic syndrome - a systematic review. *Med Sci Monit* 2012;18:RA138-44.
8. Stevens WD, Fortin T, Pappas BA. Retinal and optic nerve degeneration after chronic carotid ligation: time course and role of light exposure. *Stroke* 2002;33:1107-12.
9. Román GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. *Lancet Neurol* 2002;1:426-36.
10. Han HS, Jang JH, Jang JH, Choi JS, Kim YJ, Lee C, Lim SH, Lee HK, Lee J. Water extract of *Triticum aestivum* L. and its components demonstrate protective effect in a model of vascular dementia. *J Med Food* 2010;13:572-8.
11. Lim SH, Lee J. Hot water extract of wheat bran attenuates white matter injury in a rat model of vascular dementia. *Prev Nutr Food Sci* 2014;19:145-55.
12. Fincher GB, Sawyer WH, Stone BA. Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. *Biochem J* 1974;139:535-45.
13. Van den Bulck K, Swennen K, Loosveld AM, Courtin C, Brijs K, Proost P, Van Damme J, Van Campenhout S, Mort A, Delcour J. Isolation of cereal arabinogalactan-peptides and structural comparison of their carbohydrate and peptide moieties. *J Cereal Sci* 2005;41:59-67.
14. Lim SH, Kim MY, Lee J. Apple pectin, a dietary fiber, ameliorates myocardial injury by inhibiting apoptosis in a rat model of ischemia/reperfusion. *Nutr Res Pract* 2014;8:391-7.
15. Nishimura N, Tanabe H, Sasaki Y, Makita Y, Ohata M, Yokoyama S, Asano M, Yamamoto T, Kiriya S. Pectin and high-amylose maize starch increase caecal hydrogen production and relieve hepatic ischaemia-reperfusion injury in rats. *Br J Nutr* 2012;107:485-92.
16. Wakita H, Tomimoto H, Akiguchi I, Kimura J. Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. *Acta Neuropathol* 1994;87:484-92.
17. Farkas E, Donka G, de Vos RA, Mihály A, Bari F, Luiten PG. Experimental cerebral hypoperfusion induces white matter injury and microglial activation in the rat brain. *Acta Neuropathol* 2004;108:57-64.
18. Tykhomyrov AA, Pavlova AS, Nedzvetsky VS. Glial fibrillary acidic protein (GFAP): on the 45th anniversary of its discovery. *Neurophysiology* 2016;48:54-71.
19. Imai Y, Kohsaka S. Intracellular signaling in M-CSF-induced microglia

- activation: role of Iba1. *Glia* 2002;40:164-74.
20. Farkas E, Luiten PG, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* 2007;54:162-80.
 21. López-Valdés HE, Martínez-Coria H. The role of neuroinflammation in age-related dementias. *Rev Invest Clin* 2016;68:40-8.
 22. Rosenberg GA, Bjerke M, Wallin A. Multimodal markers of inflammation in the subcortical ischemic vascular disease type of vascular cognitive impairment. *Stroke* 2014;45:1531-8.
 23. Liu Z, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. *Prog Neurobiol* 2016;144:103-20.
 24. Saggiu R, Schumacher T, Gerich F, Rakers C, Tai K, Delekate A, Petzold GC. Astroglial NF- κ B contributes to white matter damage and cognitive impairment in a mouse model of vascular dementia. *Acta Neuropathol Commun* 2016;4:76.
 25. McDougal DH, Gamlin PD. Autonomic control of the eye. *Compr Physiol* 2015;5:439-73.
 26. Lavinsky D, Arterni NS, Achaval M, Netto CA. Chronic bilateral common carotid artery occlusion: a model for ocular ischemic syndrome in the rat. *Graefes Arch Clin Exp Ophthalmol* 2006;244:199-204.
 27. Gunes A, Demirci S, Umul A. Vision loss and RNFL thinning after internal carotid artery occlusion and middle cerebral artery infarction. *Acta Inform Med* 2014;22:413-4.
 28. Klijn CJ, Kappelle LJ. Haemodynamic stroke: clinical features, prognosis, and management. *Lancet Neurol* 2010;9:1008-17.
 29. Pretegianni E, Rosini F, Dotti MT, Bianchi S, Federico A, Rufa A. Visual system involvement in CADASIL. *J Stroke Cerebrovasc Dis* 2013;22:1377-84.
 30. Parisi V, Pierelli F, Coppola G, Restuccia R, Ferrazzoli D, Scassa C, Bianco F, Parisi L, Fattapposta F. Reduction of optic nerve fiber layer thickness in CADASIL. *Eur J Neurol* 2007;14:627-31.
 31. Rufa A, Malandrini A, Dotti MT, Berti G, Salvadori C, Federico A. Typical pathological changes of CADASIL in the optic nerve. *Neurol Sci* 2005;26:271-4.
 32. Fabri M, Pierpaoli C, Barbaresi P, Polonara G. Functional topography of the corpus callosum investigated by DTI and fMRI. *World J Radiol* 2014;6:895-906.
 33. Paul LK, Erickson RL, Hartman JA, Brown WS. Learning and memory in individuals with agenesis of the corpus callosum. *Neuropsychologia* 2016;86:183-92.
 34. Tomimoto H, Lin JX, Matsuo A, Ihara M, Ohtani R, Shibata M, Miki Y, Shibasaki H. Different mechanisms of corpus callosum atrophy in Alzheimer's disease and vascular dementia. *J Neurol* 2004;251:398-406.
 35. Jung WB, Mun CW, Kim YH, Park JM, Lee BD, Lee YM, Moon E, Jeong HJ, Chung YI. Cortical atrophy, reduced integrity of white matter and cognitive impairment in subcortical vascular dementia of Binswanger type. *Psychiatry Clin Neurosci* 2014;68:821-32.
 36. Lin L, Xue Y, Duan Q, Sun B, Lin H, Chen X, Luo L, Wei X, Zhang Z. Microstructural white matter abnormalities and cognitive dysfunction in subcortical ischemic vascular disease: an atlas-based diffusion tensor analysis study. *J Mol Neurosci* 2015;56:363-70.
 37. Best T, Howe P, Bryan J, Buckley J, Scholey A. Acute effects of a dietary non-starch polysaccharide supplement on cognitive performance in healthy middle-aged adults. *Nutr Neurosci* 2015;18:76-86.
 38. Lim SH, Kim Y, Yun KN, Kim JY, Jang JH, Han MJ, Lee J. Plant-based foods containing cell wall polysaccharides rich in specific active monosaccharides protect against myocardial injury in rat myocardial infarction models. *Sci Rep* 2016;6:38728.
 39. Zhang P, Zhang Q, Whistler RL. L-arabinose release from arabinoxylan and arabinogalactan under potential gastric acidities. *Cereal Chem* 2003;80:252-4.
 40. Bach Knudsen KE. Microbial degradation of whole-grain complex carbohydrates and impact on short-chain fatty acids and health. *Adv Nutr* 2015;6:206-13.
 41. Koropatkin NM, Cameron EA, Martens EC. How glycan metabolism shapes the human gut microbiota. *Nat Rev Microbiol* 2012;10:323-35.
 42. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 2015;161:264-76.
 43. Fitzpatrick A, Roberts A, Witherly S. Larch arabinogalactan: a novel and multifunctional natural product. *Agro Food Ind Hi Tech* 2004;15:30-2.
 44. Dion C, Chappuis E, Ripoll C. Does larch arabinogalactan enhance immune function? A review of mechanistic and clinical trials. *Nutr Metab (Lond)* 2016;13:28.
 45. Groman EV, Enriquez PM, Jung C, Josephson L. Arabinogalactan for hepatic drug delivery. *Bioconjug Chem* 1994;5:547-56.
 46. Robinson RR, Feirtag J, Slavin JL. Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects. *J Am Coll Nutr* 2001;20:279-85.
 47. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 2016;7:27-31.