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REVIEW

Neuroprotective Potential of Brown Seaweed Phytochemicals in Rodent Models of Cerebral Ischemia

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ABSTRACT Cerebral ischemia, a condition with insufficient blood flow in the brain, is associated with cognitive and behavioral changes. The underlying cellular mechanisms of ischemia-induced brain damage include oxidative stress and inflammation. Cerebral ischemia is a major cause of death and long-term disability; thus, investigating novel dietary sources and their therapeutic potentials have gained interest. Seaweed contains various functional phytochemicals with antioxidant and anti-inflammatory effects. Studies have reported that consumption of seaweed is negatively associated with the risk of cardiovascular disease and stroke in humans, but the cellular mechanisms of seaweed's effects are less known. In this review, we discuss the neuroprotective roles of seaweed phytochemicals in various models of cerebral ischemia. We further describe the potential cellular mechanisms such as the effect of seaweed phytochemicals in ischemia-mediated oxidative stress and inflammation. Additional preclinical studies are needed to develop effective dietary interventions for the prevention of ischemia-associated brain damage in humans.

KEYWORDS: • *brain* • *ischemia* • *seaweed*

INTRODUCTION

Cerebral ischemia is a condition that results from reductions in blood flow to the brain. There are two types of ischemia. Focal cerebral ischemia is caused by obstruction of blood flow to a specific area of the brain, which commonly occurs during stroke, whereas global cerebral ischemia results from the blockage of blood supply to the whole brain, which is frequently brought on by cardiac arrest.[1] According to the American Heart Association, the incidence of sudden cardiac arrest in the United States was 356,000 with a survival rate of only 9%, and the incidence of stroke was 7.6 million accounting for 1 in every 19 deaths annually in 2022.[2] The brain is an energy-demanding organ. Neurotransmission and neurodevelopment require high metabolic activities. The brain uses ~20% of the body's oxygen to produce energy.[3] Thus, limited oxygen and glucose delivery during cerebral ischemia challenges the brain to meet its metabolic demands.

In addition, cerebral ischemia triggers the production of reactive oxygen species (ROS), release of calcium, and activation of signaling cascades including excitotoxic, apoptotic, and inflammatory pathways, which damage brain cells.[4]

Neurons in the adult brain are mostly postmitotic; therefore, the brain is vulnerable to irreversible damage. Despite advances in surgical and pharmacological approaches, there has been an increasing interest in preventative strategies aligned with lifestyle. Addition of seaweeds in the diet may be preventive against ischemia-induced brain damage. Seaweeds are rich in phytosterols, polyphenols, carotenoids, and polysaccharides, which have antioxidant, anti-inflammatory, and neuroprotective properties.[5] They are commonly consumed in East Asia. Although there are limited studies demonstrating neuroprotective properties of seaweed in the Western diet, seaweed intake has been inversely associated with the risk of cardiovascular diseases including stroke in Japanese populations.[6,7]

The direct role of seaweed in cerebral ischemia in humans is less known. However, increasing studies with rodent models that mimic cerebral ischemia have suggested mechanisms of seaweed-mediated neuroprotection. In particular, middle cerebral artery occlusion (MCAO) and

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bilateral common carotid artery occlusion (CCAO) in rodents are well-established *in vivo* models to demonstrate focal and global ischemia, respectively. In this review, we investigate the effects of seaweed on various cerebral ischemia models and suggest cellular mechanisms that are potentially associated with neuroprotection.

OVERVIEW OF BROWN SEAWEED PHYTOCHEMICALS

Brown seaweed (*Phaeophyta*) is a type of algae that includes *Undaria pinnatifida, Saccharina japonica, Fucus vesiculosus*, and *Laminaria* sp.[8,9] Brown seaweed is commonly consumed as a part of the diet in East Asia. Although other classes of seaweed such as green seaweed (*Chlorophyta*) and red seaweed (*Rhodophyta*) contain various nutrients, brown seaweed has distinctive bioactive compounds. Polysaccharides such as fucoidan and laminarin are the major macronutrients that support membrane structure and serve as a source of energy storage in brown seaweed.[10] Although brown seaweed contains a lower level of protein compared with green and red seaweed, it has greater levels of lipids such as eicosapentaenoic acid (EPA) and phytosterol.[9,11,12]

In addition, fucoxanthin, a carotenoid that exhibits unique brown pigmentation, is rich in brown seaweed. Vitamin E, phenolic acids, and iodine are also abundant in brown seaweed. Antioxidant, anti-inflammatory, anticoagulant, and antitumor effects of seaweed phytochemicals have been reported in various disease models.[9] Recently, there has been an increasing interest in investigating the potential of seaweed's bioactive components to combat neuronal damage from cerebral ischemia (Table 1).

This study utilized search strategies to identify relevant articles from PubMed. The search terms used were selected to reflect the focus of the study and included keywords such as brown seaweed, brown algae, algal polysaccharide, fucoxanthin, fucoidan, laminarin, alginate, EPA, cerebral ischemia, ischemic stroke, ischemic injury, two vessel occlusion, middle cerebral artery occlusion, common carotid artery occlusion, rodent model, *in vivo,* brain, neurons, neuronal death, and cognition. Eligibility criteria for inclusion in the study required that the studies used one of the interventions of interest in *in vivo* rodent model of cerebral ischemia and were published between 2000 and 2023. Clinical studies and studies with other neurodegenerative disease models such as Alzheimer's disease and Parkinson's disease were excluded. Two authors independently screened article titles and abstracts, and then full-text sources that met the inclusion criteria were included in this review.

Fucoidan

Fucoidan is a sulfated polysaccharide with various sugar molecules including fucose, galactose, and mannose. Fucoidan is extracted from brown seaweed. Especially, *U. pinnatifida* (miyeok in Korean and wakame in Japanese) and *S. japonica* (dasima in Korean and kombu in Japanese), commonly consumed in Korea and Japan as a side dish, a soup, or a snack, are rich in fucoidan. The chemical structure of fucoidan varies by the monosaccharide ratio, sulfate content, or the molecular weight,[13] which potentially influence its bioactivity. Fucoidan exhibits antioxidant capacities including hydroxy radical scavenging activity and ferric reducing activity,[14] and treatment with fucoidan prevents oxidative stress–associated cellular damage by increasing antioxidant enzymes such as catalase and superoxide dismutase (SOD).[15] In addition, fucoidan has demonstrated anticoagulant and anti-inflammatory effects.[16] These cellular and molecular functions of fucoidan may ameliorate the pathological process of cerebral ischemia.

Kim et al. investigated the effect of fucoidan administration on global cerebral ischemia in 6-month-old male Mongolian gerbils.[17] Animals were treated with vehicle, 25, or 50 mg/kg of fucoidan followed by sham or CCAO procedure. The CCAs are the major vessels that supply blood to the brain; therefore, experimental occlusion of CCA blocks circulation in the whole brain mimicking global cerebral ischemia. Animals in the intervention groups were subjected to an intraperitoneal injection of 25 or 50 mg/kg fucoidan extract from *Fucus vesiculus* for 5 days. Global ischemia was induced by CCAO for 5 min.

The effect of fucoidan treatment was observed on locomotor activity, neuronal death, neuroinflammation, and oxidative stress. The higher dose of fucoidan (50 mg/kg) pretreatment prevented global ischemia-induced hyperactivity in animals. Loss of CA1 pyramidal hippocampal neurons is well characterized in rodent models after induction of global ischemia.[18–20] Animals treated with 50 mg/kg fucoidan retained NeuN signal and decreased Fluoro-Jade signal in the CA1 region of the hippocampus indicating protection against global ischemia-associated neuronal loss and neurodegeneration, respectively. Although the mechanisms of fucoidan-mediated neuronal protection in global ischemia are less known, treatment with fucoidan was previously reported to decrease intracellular calcium levels by regulating the N-methyl-D-aspartate receptor in primary hippocampal neurons.[21] Thus, fucoidan may prevent ischemia-induced excitotoxicity.

Kim et al. further reported that glial fibrillary acidic protein (GFAP)–positive astrocyte and ionized calcium-binding adaptor molecule 1 (IBA1)–positive microglia were decreased in the fucoidan-treated group. Because astrocyte and microglia contribute to oxidative stress and inflammation in the brain, they further assayed oxidative stress markers such as 4-hydroxynoneal (HNE) and dihydroethidium (DHE). Animals treated with fucoidan before ischemia demonstrated significantly decreased HNE and DHE-positive signals indicating attenuation of ischemia-associated lipid peroxidation and ROS production, respectively. Kim et al. further quantified the abundance of SOD1 and SOD2 protein by applying immunohistochemistry and immunoblotting. Fucoidan-treated animals were resistant to the loss of SOD1 and SOD2 after global ischemia. However, administration of diethyldithiocarbamate, an inhibitor of SOD, abolished the neuroprotective effect of fucoidan suggesting the regulatory role of fucoidan on SOD enzymes.

Similarly, Ahn et al. demonstrated the neuroprotective effect of fucoidan in obese gerbils with global cerebral

Table 1. List of *In Vivo* Studies Investigating the Role of Seaweed Phytochemical in Cerebral Ischemia Models

Phytochemical	*Treatment*	*Animal*	*Cerebral ischemia*	*Evaluation*	*Ref*
Fucoidan	25 and 50 mg/kg, intraperitoneal	Male Mongolian gerbil, 6 months old	Global ischemia	Neuronal death Lipid peroxidation and ROS Inflammation Locomotor function	Kim et al.[17]
Fucoidan	50 mg/kg, intraperitoneal	Male Mongolian gerbil, 6 months old	Global ischemia	Neuronal death Lipid peroxidation and ROS DNA oxidation Antioxidant enzymes	Ahn et al.[22]
Fucoidan	80 and 160 mg/kg, intraperitoneal	Male Sprague-Dawley rats, 250–320 g	Focal ischemia	Infarct volume Inflammation Antioxidant enzymes Lipid peroxidation Apoptosis MAPK pathway Neurological function	Che et al.[32]
Laminarin	50 mg/kg, intraperitoneal	Male Mongolian gerbil, 22–24 months old	Global ischemia	Neuronal death Lipid peroxidation and ROS Antioxidant enzymes Inflammation	Park et al.[43]
Laminarin	10, 50 and 100 mg/kg, intraperitoneal	Male Mongolian gerbil, 6 months old	Global ischemia	Neuronal death Inflammation	Lee et al.[45]
Laminarin	10 mg/kg, intraperitoneal	Male Sprague-Dawley rats, 9 weeks old	Focal ischemia	Infarct volume Bioinformatic analysis Neurological function	Luo et al.[46]
Fucoxanthin	30, 60 and 90 mg/kg, intragastric	Male Wistar rats	Focal ischemia	Infarct volume Brain edema Lipid peroxidation and ROS Apoptosis Nrf2/HO1 pathway Neurological function	Hu et al.[58]
EPA	500 mg/kg, intraperitoneal	Male Mongolian gerbil, 13–15 weeks old	Global ischemia	Neuronal death DNA oxidation Inflammation Memory function	Okabe et al.[61]
EPA	30, 100 and 300 mg/kg, oral gavage	Male Sprague-Dawley rats, 250–300 g	Focal ischemia	Plasma EPA and arachidonic acid quantification Lipid peroxidation DNA oxidation Infarct volume Rho-kinase activation von Willebrand factor Neurological function	Ueda et al.[62]
EPA	20 and 30 mg/kg, oral via a gastric tube	Male C57BL/6, GRP40$^{-/-}$, GRP120 $^{-/-}$ mice, 8 weeks old	Focal ischemia	Infarct volume Inflammation Apoptosis Neurological function	Mo et al.[63]

EPA, eicosapentaenoic acid; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species.

ischemia.[22] Obesity is a risk factor for cerebral ischemia, and obesity is known to exacerbate ischemia-associated brain injury.[23,24] Male Mongolian gerbils (6 months old) were fed a normal-fat or high-fat diet for 12 weeks, and 50 mg/kg fucoidan was intraperitoneally administrated for the last 5 days during high-fat diet exposure. Then, animals were subjected to 5 min of global ischemia induced by CCAO, and the time-course analysis was performed 1, 2, and 5 days after global ischemia. Animals that underwent CCAO showed the loss of CA1 pyramidal neurons in the hippocampus. The hippocampal CA2 and CA3 regions are normally resistant to ischemia-associated damage.[25] However, neurons in the CA2 and CA3 regions of the hippocampus lost their morphology in the high-fat diet group.

Consistent with Kim et al.,[17] NeuN and Fluoro-Jade staining demonstrated that treatment with fucoidan prevented global ischemia-induced neuronal loss in CA1. Their results indicate fucoidan was effective to protect the CA2 and CA3 regions of the hippocampus in gerbils subjected to a high-fat diet followed by global ischemia. Ahn et al. also found that treatment with fucoidan prevents high-fat diet-associated ROS production, DNA oxidation, and lipid peroxidation in global ischemia evaluated by DHE, 8-hydroxyguanosine, and DHE staining, respectively. In

addition, obese gerbils injected with fucoidan retained greater levels of SOD1 and SOD2 proteins in their hippocampus after an ischemic event. Together, these findings suggest potential neuroprotective properties of fucoidan in obese populations with a high risk of global cerebral ischemia.

Oxidative stress is one of the major contributors to ischemia-induced damage in the brain, and both studies highlighted the effect of seaweed for attenuating ROS production or preventing the loss of antioxidant enzymes in global ischemia.[17,22] Treatment with fucoidan prevents ischemia-induced lipid peroxidation, superoxide accumulation, and DNA oxidation in rodent models.[17,22] Fucoidan is previously reported to exhibit a strong free radical scavenging activity.[26,27] However, it is unclear if intravenously introduced fucoidan can be directly delivered to the brain. Of interest, researchers suggest that fucoidan may regulate receptor-mediated cell signaling pathways.[28] Both studies showed that treatment with fucoidan prevents the loss of SOD1 and SOD2 in global ischemia.[17,22]

Although there are limited studies demonstrating the mechanisms of transcriptional or translational regulation of SODs in fucoidan treatment, fucoidan is reported to increase the protein level of nuclear factor erythroid 2–related factor-2 (Nrf-2) in the nuclear fraction *in vitro*.[15,29] Because the antioxidant response element (ARE) is found in the promoter region of SODs, and Nrf-2 binds to ARE upregulating SOD genes,[30,31] treatment with fucoidan may increase protein levels of SOD via transcriptional regulation.

In addition to global cerebral ischemia, Che et al. investigated the effects of fucoidan in focal cerebral ischemia using Sprague-Dawley rats that underwent MCAO.[32] Fucoidan (80 or 160 mg/kg) was intraperitoneally injected 7 days before MCAO until being killed. Neurological deficits were evaluated at 24 h after MCAO. Animals that underwent MCAO showed partial paralysis on the affected side, but treatment with fucoidan before MCAO significantly decreased the neurological deficit scores indicating improved motor function. TTC staining showed that both 80 and 160 mg/kg fucoidan reduced infarct volume in the MCAO-induced brain. Fucoidan treatment prevented MCAO-induced MPO levels indicating inhibition of neutrophil infiltration in the brain tissue. Consequently, levels of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β were decreased in the fucoidan-treated group.

Because inflammation leads to oxidative stress, Che et al. quantified malondialdehyde (MDA), a marker of lipid peroxidation, and SOD. Ischemia increased both MDA and SOD levels, whereas treatment with fucoidan before MCAO significantly decreased MDA and SOD. Che et al. further explored cellular mechanisms of ischemia-mediated cellular damage. Treatment with fucoidan increased the abundance of anti-apoptotic Bcl-2 and decreased pro-apoptotic Bax in focal ischemia, suggesting the mitochondrial apoptotic pathway may be an important target.[32] Mitochondria-mediated apoptotic pathway is well-described in cerebral ischemia. Ischemic insult stimulates activation of Bax, and oligomerization of Bax in the mitochondrial membrane increases membrane permeability.[33,34] The loss of mitochondrial membrane integrity contributes to cytochrome c release followed by apoptosome formation and caspase activation. Strategies to prevent Bax activation have shown neuroprotective properties in both *in vivo* and *in vitro* cerebral ischemia models.[35,36]

In addition, approaches that protect anti-apoptotic proteins such as Bcl-2 and Bcl-xL are neuroprotective by preventing mitochondrial dysfunction.[18,36] Furthermore, Che et al. explored the role of fucoidan in the MAPK pathway.[32] Treatment with fucoidan prevents the abundance of phosphorylated p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) protein in ischemia-induced brain damage. The MAPK pathway including p38 and JNK was previously reported to promote mitochondrial translocation of Bax in other pathology models[37–39]; thus, it is possible that fucoidan may inhibit the abundance of mitochondrially localized Bax-supporting neurons to maintain mitochondrial membrane integrity.

Laminarin

Laminarin is a low–molecular weight β-1, 3-glucan found in brown seaweed such as *Laminaria hyperborea, Laminaria digitata*, and *F. vesiculosus*.[40,41] Laminarin forms β-1, 6-branches with other sugar molecules such as glucose and mannitol. The structure of laminarin including the ratio of the side chain and the degree of polymerization is influenced by the species of seaweed and the extraction conditions. The level of laminarin in brown seaweed is also affected by the season of collection. Laminarin exhibits anticancer properties by inhibiting cancer cell survival, colony formation, and angiogenesis.[42] Although there are limited data elucidating the roles of laminarin in brain-associated diseases, recent studies demonstrated the neuroprotective properties of laminarin in global cerebral ischemia.

Park et al. intraperitoneally injected 50 mg/kg laminarin followed by 5 min global ischemia.[43] Of interest, this group used 22- to 24-month-old Mongolian gerbils to demonstrate the effect of laminarin in the aged brain. Treatment with 50 mg/kg laminarin rescued CA1 hippocampal neurons against global ischemia. Park et al. quantified protein levels of cytokines using immunohistochemistry. Global ischemia increased proinflammatory cytokines such as IL-1β and TNF-α, but decreased anti-inflammatory cytokines such as IL-4 and IL-13. Treatment with laminarin prevented ischemia-mediated changes in cytokine levels. Park et al. further conducted time-course measurement of oxidative stress markers. Laminarin was previously shown to scavenge free radicals by direct antioxidant properties.[44] Park et al. showed that global ischemia increases ROS production and lipid peroxidation at 1 and 5 days after global ischemia, whereas treatment with laminarin prevented ischemia-induced oxidative stress in the brain.

Similarly, animals pretreated with laminarin were more resistant to the loss of antioxidant enzymes SOD1 and SOD2 after global ischemia. Of interest, the antioxidant properties

may be influenced by purification methods and chemical composition of laminarin. Therefore, investigating different types of seaweed extract may be important to develop therapeutic strategies in the future.

Lee et al. intraperitoneally injected vehicle or laminarin (10, 50, or 100 mg/kg) for 7 days in 6-month-old male Mongolian gerbils. Then, injections were followed by 5 min of global ischemia.[45] Lee et al. found concentration-dependent neuroprotection in laminarin treatment. Mongolian gerbils injected with a lower concentration, 10 mg/kg, before global ischemia showed the loss of CA1 pyramidal neurons after ischemia. However, treatment with higher concentrations including 50 and 100 mg/kg prevented the ischemia-induced neuronal loss in the hippocampus evaluated by cresyl violet, NeuN, and Fluoro-Jade staining. To assess neuroinflammation-mediated neuronal death, activated astrocytes and microglia were visualized by applying GFAP and IBA-1 staining, respectively. Intraperitoneal injection of 50 and 100 mg/kg laminarin prevented ischemia-mediated expression of GFAP and IBA-1. Lee et al. further performed co-immunostaining of IBA-1 and IL-2. Although there is no quantitative analysis, microglia in the ischemia group were colocalized with IL-2, but IL-2 fluorescence was decreased in the laminarin-treated group.

Recently, Luo et al. performed comparative transcriptome sequencing and bioinformatic analysis in rat focal ischemia models.[46] Sprague-Dawley rats were subjected to 2 h MCAO or sham followed by reperfusion. Then, animals underwent intraperitoneal injection of laminarin (10 mg/kg) or saline for 7 days. Treatment with laminarin significantly decreased the infarct volume, and improved neurological scores evaluated by motor, sensory, balance, and reflex tests in animals with MCAO procedures. Their transcriptomic profiles showed that treatment with laminarin changed 957 genes in the brain. Of note, genes that control inflammatory responses, apoptosis, nervous system development, neuronal differentiation, calcium signaling, and death receptor signaling were highly regulated by laminarin. The mechanisms shown in global ischemia models such as inhibition of pro-inflammatory cytokines and activation of microglia in the laminarin-treated group may be attributed to its transcriptional regulation in immune and inflammatory responses.

In addition, laminarin regulates genes that control cell migration, vascular permeability, and blood vessel development in MCAO-induced brain, suggesting the beneficial effect during recovery after ischemia. Although it is unknown whether the protective effects of laminarin would persist over a longer period of time, this group showed that treatment with laminarin alleviated MCAO-mediated neurological deficit up to 7 days.

Fucoxanthin

Fucoxanthin is a marine carotenoid belonging to the xanthophyll family found mainly in brown algae such as *U. pinnatifida*,[47] *Laminaria japonica*,[48] *Sargassum fusiformis*,[49] *F. vesiculosus*,[50] and *Hijikia fusiformis*.[51] Fucoxanthin is rapidly hydrolyzed in the gastrointestinal tract into fucoxanthinol, which is then converted into amarouciaxanthin A.[52] One study showed that the highest concentration of fucoxanthinol and amarouciaxanthin A was detected 2 h after a single oral administration of fucoxanthin in the plasma and the liver of mice.[52] Mice that underwent 1 week of oral administration showed both fucoxanthin and its metabolites in various tissues including liver, lung, kidneys, and adipose tissues.[53] A toxicity study showed that no mortality was found among mice treated with a single dose of 1000 and 2000 mg/kg and chronic administration of 500 and 1000 mg/kg for 30 days.[54] This study also demonstrated no significance in fucoxanthin-associated body weight changes in male and female animals.

Fucoxanthin exhibits antioxidant properties by scavenging free radicals. Fucoxanthin is reported to scavenge hydroxyl free radicals better than alpha-tocopherol.[55] Fucoxanthin also exhibits anti-inflammatory properties in glial cells by reducing levels of anti-inflammatory cytokines.[56,57] Neuroinflammation is associated with the onset of neurodegeneration. Cerebral ischemia induces inflammatory response in the brain leading to elevated ROS levels and consequently neuronal death. Fucoxanthin is a less studied component of brown algae in cerebral ischemic animal models. The capacity of fucoxanthin to cross the blood–brain barrier is not confirmed, but it has demonstrated neuroprotective activities in a few *in vivo* studies.

In a rodent model of focal cerebral ischemia, intragastrically administered dosages of 30, 60, and 90 mg/kg fucoxanthin 1 h before MCAO improved neurological deficit score and reduced brain infarct sizes in rats.[58] Immunoblotting data showed levels of apoptosis-related proteins. Fucoxanthin treatment decreased the protein levels of caspase-3 and Bax and increased anti-apoptotic Bcl-2 levels in the infarcted tissues. In addition, levels of antioxidant enzyme SOD were increased, whereas lipid peroxidation marker MDA level was decreased in the infarcted areas by fucoxanthin treatment in a dose-dependent manner. The researchers concluded that fucoxanthin exerted protective effects against ischemic brain damage possibly by targeting ischemia-induced oxidative stress.

To further explain the potential neuroprotective mechanism of fucoxanthin *in vitro,* primary cortical neuron cultures were subjected to oxygen-glucose deprivation and reperfusion to mimic ischemia after 5, 10, and 20 μM fucoxanthin were administered. Similar to the *in vivo* study, fucoxanthin treatment successfully protected the cortical neurons from apoptosis. Fucoxanthin-treated neurons also had higher expression of anti-apoptotic Bcl-2 and reduced expression of pro-apoptotic Bax proteins like fucoxanthin-treated brain tissue.

Eicosapentaenoic acid

EPA, an omega-3 polyunsaturated fatty acid, is one of the primary fatty acids found in brown seaweed. In humans, higher concentrations of EPA in serum were associated with a lower risk of Alzheimer's disease.[59] EPA has shown neuroprotective potential during cerebral ischemia in humans and animals. High blood levels of EPA have been

shown to reduce brain infarction in patients with atrial fibrillation.[60] The neuroprotective effect of EPA has been investigated in animal models of cerebral ischemia. Intraperitoneally injected 500 mg/kg of EPA for 4 weeks in Mongolian gerbils improved memory function and prevented ischemia-mediated cell death in the CA1 region of the hippocampus.[61] Further investigation showed a reduced number of activated microglia in the hippocampal CA1 area indicating reduced inflammation. EPA treatment also prevented DNA fragmentation, an apoptosis marker, in the hippocampus.

Ueda et al. investigated ethyl-EPA in a rat model of focal cerebral ischemia. Animals received 100 mg/day ethyl-EPA by oral gavage once daily for 3, 5 or 7 days before MCAO surgery.[62] Pretreatment with ethyl-EPA for 5 and 7 days decreased cortical infarct volume and improved neurological scores. Immunohistological analysis showed that pretreatment with ethyl-EPA decreased 8-OHdG, 4-HNE, and phosphorylated adducin-positive cells suggesting the protective effects of EPA against DNA oxidation, lipid peroxidation, and rho-kinase activation. Mo et al. administered in the mice via a gastric tube in 10, 20, and 30 mg/kg doses for 2 weeks before MCAO.[63] This group focused on inflammatory pathways investigating the role of NLR family pyrin domain containing 3 (NLRP3), a critical regulator of innate immunity and inflammation, under EPA treatment. Treatment with 20 and 30 mg/kg EPA prevented ischemia-associated infarct and neurological deficits and decreased protein levels of NLRP3. NLRP3 knockout animals were resistant to brain injury associated with MCAO-mediated inflammatory responses.

To further confirm the inactivation of NLPR3 by EPA, an *in vitro* oxygen-glucose deprivation model with glial cells was used. Similar to the *in vivo study,* EPA can inhibit NLPR3 activation by nigericin, an NLPR3 activator, indicated by decreased levels of IL-1β, IL-18, and inhibition of caspase-1 cleavage. G protein–coupled receptors found on the surface of immune cells play an essential role in inflammatory pathways. This study also found that EPA suppresses NLPR3 activation via GPR40 and GPR120 observed in both *in vitro* and *in vivo* models. Absence of these proteins inhibited the protective effects of EPA during ischemic injury. By tackling oxidative stress and inflammatory pathways, EPA can exhibit neuroprotective properties in global and focal ischemia models.

Alginate

Alginate is a structural edible anionic heteropolysaccharide found in the cell wall of brown seaweed.[64] It is composed of two isomers, β-D-mannuronate and α-L-glucuronate along with hydroxyl and carboxyl functional groups. Alginate exerts antioxidant and chelation activity.[65,66] Alginate content for *Ascophyllum nodosum* and *L. digitata* are 22–30% and 25–44% of dry weight, respectively, and alginate constitutes 40% of dry weight in different brown algae species.[64,67] Alginate and its derivatives

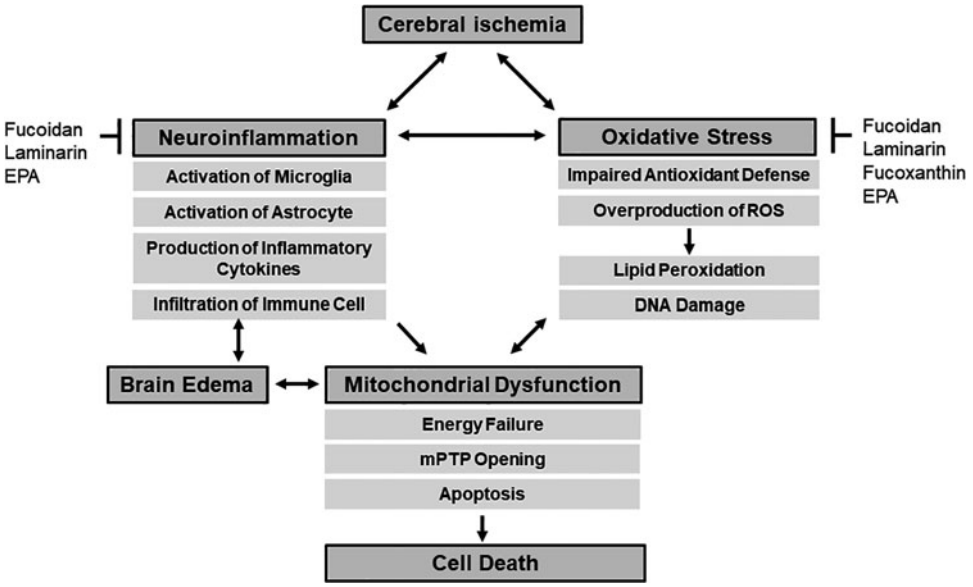

FIG. 1. Mechanisms of neuronal death during cerebral ischemia. Ischemia triggers a cascade of events that leads to brain cell death. These include inflammation, which activates microglia and astrocytes, increases in pro-inflammatory cytokines production, and inflammasome activation, and neutrophil infiltration. The overproduction of ROS and the depletion of endogenous antioxidant enzymes lead to oxidative stress, which results in lipid peroxidation, DNA damage, and protein oxidation. Inflammation and oxidative stress are interlinked processes that mutually exacerbate each other during cerebral ischemia. In addition, inflammation and oxidative stress cause mitochondrial dysfunction, which impairs ATP production worsening energy crisis-associated with ischemia-mediated oxygen and nutrient deficit. Damaged mitochondria are responsible for triggering cell death mechanisms such as mPTP opening and apoptosis. Together, these pathways contribute to the pathogenesis of neuronal death during cerebral ischemia. Studies discussed in this review demonstrated protective effects of various seaweed phytochemicals in the ischemic brain by preventing inflammation, oxidative stress, and their downstream pathways. ATP, adenosine triphosphate; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species.

exhibit protective effects against various cytotoxic challenges including oxidative stress, apoptosis, autophagy, and neurodegeneration. Biomedical applications of alginate include cell and microorganism immobilization,[68] anticoagulant therapy,[69] drug delivery,[70,71] tissue engineering,[72] wound dressing,[67] transplantation of stem cells, and tissue regeneration.[73] In addition, alginate is a hydrocolloid commonly used in the food and pharmaceutical industry as a biopolymer and gel-based agent owing to its high viscosity, stability, biocompatibility, and biodegradability.[74–76]

Neuroprotective properties of alginate have been reported in *in vitro* models. NT2 cells treated with sodium alginate followed by H_2O_2 and $FeSO_4$ challenges showed a greater cell viability than the group without alginate treatment.[77] In addition, the cells treated with alginate increased HO-1, GSH, glutamyl cysteine synthetase, and Nrf2 in H_2O_2 and $FeSO_4$ treatment suggesting resistance against redox changes. Similarly, Tusi et al. demonstrated the neuroprotective effects of alginate during H_2O_2-induced oxidative stress.[78] Treatment with alginate prevented apoptosis against oxidative stress in PC12 cells. In addition, treatment with alginate prevents H_2O_2-induced phosphorylation of p-38, JNK, and ERK, the loss of Nrf2, and the induction of NF-κB suggesting its role regulating MAPK, antioxidant, and inflammatory pathways. Although there is no *in vivo* study demonstrating the protective effect of alginate in cerebral ischemia, alginate may potentially exhibit protective effects in ischemic brain by blocking oxidative stress or apoptosis.

Of interest, Xu et al. showed the delivery of neural stem cells (NSCs) encapsulated with lipid-alginate microcapsule decreased MCAO-induced brain infarct.[79] This study also showed that lipid-alginate encapsulation enhanced NSC autophagy by inducing autolysosome formation and increased cell survival after oxygen and glucose deprivation *in vitro*. The cellular mechanisms of autophagy in lipid-alginate encapsulation are less known; however, treatment with alginate-derived oligosaccharides has been shown to decrease Tau protein aggregation by enhancing the LC3-II/LC3-I ratio and decreasing the p62 protein levels indicating increased cell autophagy.[80] Therefore, it may be important to further expand investigation of biological functions of alginate as a biodelivery tool and a neuroprotectant.

In conclusion, considering the alarming incidence of cardiac arrest and stroke,[2] cerebral ischemia is a major public health concern. Recent evidence suggests that bioactive compounds in brown seaweed may protect against cerebral ischemia-induced neuronal damage. Phytochemicals in brown seaweed such as fucoidan, laminarin, fucoxanthin, and EPA have demonstrated neuroprotective properties in *in vivo* models of focal and global ischemia (Fig. 1). The consistency of the results of the studies included in this review indicates that treatment with brown seaweed extracts protects the brain from cerebral ischemia-induced oxidative stress and inflammation in rodent models.

Further preclinical studies are needed to determine how the different phytochemicals in brown seaweed may act in synergy. Future studies can further elucidate the cellular mechanisms underlying seaweed's neuroprotective properties by examining various delivery approaches, determining the optimal timing and duration of interventions, and investigating possible effects of biological sex on the outcome measures. Nevertheless, results from recent animal studies suggest a translational potential to develop strategies for prevention of damage associated with ischemic injury in humans.

AUTHORS' CONTRIBUTIONS

K.A.F. was involved in study design, interpretation of findings, drafting the original article, and editing of the article. S.S. was involved in interpretation of findings, drafting the original article, and editing of the article. A.C.E. was involved in drafting the original article and editing of the article. H.-A.P. was involved in study design, interpretation of findings, drafting the original article, and editing of the article.

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