



Neuroprotective Properties of Omega-3 in Ischemic Cerebrovascular Accident: A Systematic Review

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Abstract: Cerebrovascular Accident (CVA) is a neurological disorder whose pathophysiology of ischemic side occurs together with the development of a central inflammatory process. Thus, omega-3 fatty acids appear as a potential agent in the prevention and treatment of the repercussions of this ischemic event. This work aims to develop a systematic review of the literature on the neuroprotective potential of omega-3 in terms of preventive and therapeutic aspects in ischemic stroke. A bibliographic review was carried out in the PubMed and Scopus electronic databases, and 12 indexed articles were included in the final sample and applied for discussion. Studies have pointed to the decrease in omega-3 as harmful to nerve tissue regeneration, as well as the neuroprotective role of omega-3 in cerebral ischemia after ischemia-reperfusion injury in animal models. Reduction of cerebral edema, promotion of angiogenesis, preservation of the integrity of the blood-brain barrier and tissue protection were also observed.

Keywords : Cerebral ischemia. Omega-3 Polyunsaturated Fatty Acids. Neuroprotection.

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Propriedades Neuroprotetoras do Ômega-3 no Acidente Vascular Cerebral Isquêmico: Uma Revisão Sistemática

Resumo: O Acidente Vascular Encefálico (AVE) é um distúrbio neurológico cuja fisiopatologia do lado isquêmico ocorre juntamente com o desenvolvimento de um processo inflamatório central. Dessa forma, os ácidos graxos ômega-3 aparecem como potencial agente na prevenção e tratamento das repercussões desse evento isquêmico. Este trabalho tem como objetivo desenvolver uma revisão sistemática da literatura sobre o potencial neuroprotetor do ômega-3 quanto aos aspectos preventivos e terapêuticos no AVC isquêmico. Foi realizada uma revisão bibliográfica nas bases de dados eletrônicas PubMed e Scopus sendo que 12 artigos indexados foram incluídos na amostra final e aplicados para discussão. Os estudos apontaram a diminuição do ômega-3 como prejudicial à regeneração dos tecidos nervosos, bem como o papel neuroprotetor do ômega-3 na isquemia cerebral após lesão de isquemia-reperfusão em modelos animais. Também foram observadas redução do edema cerebral, promoção da angiogênese, preservação da integridade da barreira hematoencefálica e proteção tecidual.

Palavras-chave: Isquemia cerebral. Ácidos graxos poliinsaturados ômega-3. Neuroproteção.

Introduction

Cerebrovascular Accident (CVA) is one of the neurological disorders with the highest mortality and permanent sequelae rates (Azarpazhooh et al., 2019). Among 240 causes of mortality, CVA is the second globally, surpassed only by ischemic heart disease (IHD), and the continuity of the rates is projected until 2030. Survivors of this morbidity may be affected by organic and psychological functional complications, requiring temporary or permanent assistance that demands high human and economic costs. Globally, the gross numbers of new stroke events increased from 6.8 million in 1990 to 11.9 million in 2017 (Avan et al., 2019).

There are two types of CVA: ischemic and hemorrhagic. Briefly, the first includes atherothrombotic cerebral infarction, cardioembolic stroke and lacunar infarct, while the second, intracerebral hemorrhage and subarachnoid hemorrhage. Both have common risk factors, such as smoking, alcoholism, chronic arterial disease and previous strokes. In addition, studies suggest the influence of some genetic component on Cerebrovascular Accident (Yamada, 2020).

Analyzing the pathophysiological pathway of CVA, the important role of neuroinflammation in the pathogenesis of ischemic stroke and the installation of brain injury

are understood. Experimentally, focal cerebral ischemia induces time-dependent recruitment and activation of inflammatory cells, including neutrophils, T cells and macrophages. The inhibition of the neuroinflammatory response decreases the extent of the infarction and provides less neurological deficit in the experimental lesion (Jin et al., 2010). Polyunsaturated fatty acids (PUFAs) are able to partially inhibit many aspects of inflammation, including leukocyte chemotaxis, expression of adhesion molecules and leukocyte-endothelial adhesive interactions, eicosanoid production and production of inflammatory substances (Calder, 2017).

Regarding the biochemical characteristics, PUFAs of the omega-3 family include α -linolenic acid (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The oils that contain these fatty acids (FAs), or some of them, originate mainly from plant sources or are modified in plants, as well as marine, algae and unicellular sources (Shahidi & Ambigaipalan, 2018).

Under the neuroprotective perspective of omega-3 PUFAs, studies have linked the effects of EPA to the suppression of oxidative stress and endothelial activation which were induced after cerebral ischemia. In this context, there is an evaluation of the anti-inflammatory and antinociceptive actions of omega-3 polyunsaturated fatty acid (n-3 PUFA) whose suggested mechanisms may involve the inhibition of cyclooxygenases and microglial activation, leading to a reduced release of pro-inflammatory cytokines, such as TNF- α (Nobre et al., 2013).

Later studies by Nobre et al., (2016) also demonstrated that the administration of low doses (5mg and 10mg/kg) of omega-3 caused a protective effect in rats against global ischemic injuries, a potential attributed to their anti-inflammatory properties (Nobre et al., 2016). This article, therefore, aims to conduct a systematic review of the literature on the neuroprotective potential of omega-3 in ischemic CVA, given the important findings that can contribute to mitigate the effects of this serious public health problem.

Methodology

A systematic review was carried out in the electronic databases PubMed and Scopus to select published studies on the neuroprotective potential of omega-3 in the prevention or treatment of ischemic stroke, presenting epidemiological features, clinical and laboratory manifestations and pharmacological relationships following the items of Guidelines for

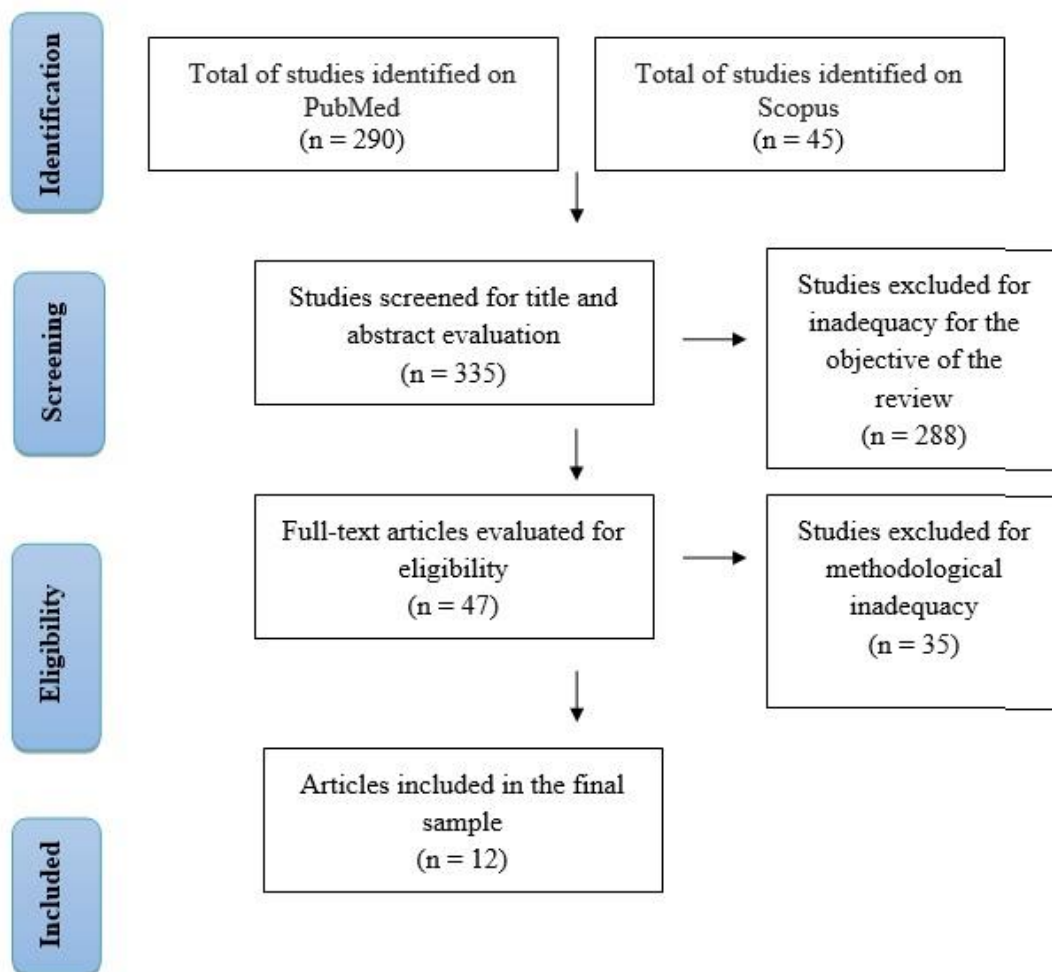
Systematic Reviews and Meta-analyzes (PRISMA). There were two independent reviewers and a third reviewer consulted in cases of articles of conflicting interest.

The keywords “ cerebral ischemia ”; “ Stroke”; “Omega-3 fatty acids; “DHA”; “EPA”; “Neuroprotection” and “neurological function” were applied to identify articles published between January 2015 and March 2020. The inclusion criteria were studies published in English, suitability for the purpose of this review, methodological rigor applied and full-text available for free. Review articles, as well as comments on literature, editorials, communications and letters to the editor were excluded. Each article was read in its entirety and its information was arranged in a spreadsheet in the Microsoft Excel program, including year of publication, authors, database, journal, country of study, objective, methodology, results and conclusion. Subsequently, the main findings of each article selected in the final sample were compiled in this review, being discussed according to the literature.

Results

A total of 335 articles were designated for screening using the search strategy. After screening by title and abstract, 47 articles were selected for full-text evaluation. Out of these studies, 35 were excluded due to the unsuitability for the objective of this review. Thus, 12 articles were included for study and discussion in the literature (Figure 1). The main characteristics of the included studies are presented in Table 1. This review included 12 studies published between 2015 and 2017, carried out between Brazil, China, the United States, Germany, South Korea, France and Japan (Table 1).

Figure 1. Flowchart summarizing the search strategy for studies.



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Table-1. Author and year of publication, country of study, objective, methodology, results and conclusion of the 12 studies included.

Author and year	Country	Objective	Methodology	Results	Conclusion
Zhang et al. (2015)	China; United States	To elucidate the mechanisms underlying the protection afforded by n-3 polyunsaturated fatty acids (n-3 PUFAs) against neuronal ischemic injuries.	N-3 PUFAs supplementation was started on the 2nd day of gestation in rats. Hypoxia-ischemia (HI) damage was induced in 7-day-old puppies by occlusion of the ipsilateral common carotid artery followed by hypoxia. Loss of brain tissue, cell death, and activation of signaling events were assessed after HI. The effects of n-3 PUFAs on cell death induced by oxygen-glucose deprivation and the underlying protection mechanism were also examined	N-3 PUFAs reduced brain tissue loss at 7 days after HI and improved neurological outcomes, while inhibition of PI3K/Akt signaling partially canceled out this neuroprotective effect. DHA/EPA prevented ischemic neuronal death and increased phosphatidylserine production.	N-3 PUFAs protect against brain damage induced by HI in neonates, activating the Akt pathway of survival in compromised neurons, promote the formation of phosphatidylserine and promote Akt activity.
Shi et al. (2015)	China; United States	To verify the effect of omega-3 polyunsaturated fatty acids (n-3 PUFAs) on the increased survival of cortical neurons cultured in the oxygen-glucose deprivation/reperfusion (OGD/R) injury model.	Rats were fed a diet containing 10% corn oil, for a period of up to 10 weeks. Cell culture and OGD/R, fatty acid analysis, biochemical, immunocytochemical and histopathological tests, TUNEL model, intracellular ROS evaluation in vivo were used. The statistics were made using the ANOVA and Turkey tests. An ischemic model with reproducible cortical infarction and function deficits was also used. Gait analysis and adhesive removal test were also performed.	Fat-1 neurons exhibited significantly attenuated reactive oxygen species (ROS) activation induced by OGD / R injury, increased presence of anti-apoptotic proteins Bcl-2 and Bcl-xL, and reduced cleavage of caspase-3. Exogenous administration of docosahexaenoic acid (DHA), resulted in similar protective effects on neurons in the cultured cortex.	Therefore, we provide evidence that n-3 PUFA exert its protective effects against ischemic injury both in vitro and in vivo, partially by inhibiting ROS activation.
Sumiyochi et al (2015)	Japan	To verify if HMG-1 plays a role in ischemic brain damage in ovariectomized rats (OVX +) and if eicosapentaenoic acid (EPA) inhibits the activation of this pathway and attenuates brain damage	Seven-week-old Sprague-Dawley rats, divided into 3 groups were used; non-ovariectomized rats (OVX-) and OVX + rats treated with EPA and not treated with EPA before induction of cerebral ischemia. Another set of OVX + mice treated with EPA was injected with the inhibitor of Peroxisome Proliferator-Activated Receptor gamma (PPAR γ) antagonist, GW9662.	The rats (OVX +) reduced the level of PPAR γ mRNA and increased that of HMG-1, receptor for advanced glycation end products (RAGE), toll-like receptor 9 (TLR9) and tumor necrosis factor alpha (TNF α) simultaneously with brain damage. EPA restored PPAR γ expression, down-regulated molecules	The volume of cortical infarction suffered by OVX + is associated with positive regulation of the HGM-1/TLR9 pathway. Suppression of this pathway can help limit this damage to postmenopausal women.

		in a PPAR γ -dependent manner.		related to the HMG-1 signal and reduced brain damage.	
Lin et al. (2015)	China; United States	To examine whether the docosahexaenoic acid (DHA) can protect the brain from injury and decrease the risk of hemorrhagic transformation (HT) increased by hyperglycemia after focal ischemia.	A substance with 50% dextrose (6ml / kg intraperitoneally) was injected into male Sprague-Dawley rats to induce hyperglycemia 10 min before 1.5 hours of occlusion of the middle cerebral artery (MCAO) of the filament combined with treatment with DHA (10mg / kg) 5min before reperfusion.	Treatment with DHA 5 min before reperfusion reduced HT and improved the neurological outcome of 7 days. It reduced the volume of the infarction and improved the integrity of the blood-brain barrier (BBB) in rats treated with DHA. In addition, DHA reduced the expression of intercellular adhesion molecule-1 (ICAM-1) in the brain.	DHA attenuated hyperglycemia and improved neurological function, preserving the integrity of the BBB and reducing inflammation.
Song et al. (2015)	South Korea	To verify the hypothesis that a low proportion of plasma fatty acids (FA) would be associated with cerebral small vessel diseases (CSVD).	Study with 220 patients with first-episode cerebral infarction, up to 7 days after the onset of symptoms. The composition of plasma FAs was analyzed using gas chromatography. The presence and load of cerebral micro-hemorrhages (CMH), high degree of white matter changes (WMC), enlarged perivascular spaces (EPVS) and asymptomatic lacunar infarctions (ALI) were investigated	CMHs, WMCs and EPVSs were negatively correlated with the proportion of EPA, DHA and n-3 PUFA in the univariate analysis; as for multivariate, a lower proportion of EPA, DHA and n-3 PUFAs was associated with the presence of CMHs, WMCs and EPVS, but not ALIs. The total score of CSVD was inversely correlated with the proportion of EPA, DHA and n-3 PUFA.	An association was found between low proportions of plasmids and n-3 PUFA and cerebral CSVD pathologies.

Bourourou Heurteaux & Blondeau (2016)	France	To evaluate the use of alpha-linolenic acid (ALA) as adjunctive therapy for stroke recovery, comparing whether oral or intravenous AAL supplementation would best assist in the recovery from ischemia.	Male rats, 4 weeks old, with a diet enriched with ALA were used, the lipids from rapeseed oil represent 10% and AAL, 0.75% by weight. Body weight and water and food consumption were monitored. Regarding parenteral supplementation, the animals were injected into the penile vein 2 h and 3, 7, 10, 14, 17 and 21 days after the middle cerebral artery occlusion (MCAO).	ALA supplementation in the diet was better than intravenous treatment in improving motor coordination, but this improvement was not due to a neuroprotective effect, since the size of the infarction was not reduced. Both types of supplementation improved spatial learning and memory after a stroke. This cognitive improvement correlated with the longer survival of neurons in the hippocampus.	It was identified that the preservation of the hippocampus has an intrinsic effect in the treatment with ALA, leading to reduced cognitive deficits, which is not due to the bioconversion of DHA.
Berressem et al (2016)	Germany	To test the effectiveness of an acute treatment with a long-chain omega-3 lipid emulsion (OGV), containing fish oil and α -tocopherol in a transient middle cerebral artery occlusion (MCAO) model of ischemic stroke.	Female mice were subjected to 90 minutes of MCAO. A single dose of OGV is applied after stroke or reperfusion induction. Motor function, neurological outcome and stroke-related parameters were assessed 24 hours after MCAO. Samples were collected from the extracellular space of the striatum. Mitochondrial function was determined in isolated mitochondria or dissociated brain cells and inflammation markers were measured in cerebral homogenate.	Intravenous OGV injection reduced stroke size and severity, restored mitochondrial function and prevented the release of excitotoxic glutamate. The increase in pro-inflammatory markers was attenuated, the neurological severity score and neurochemical data demonstrated that the acute treatment with OGV immediately after stroke induction was more efficient and capable of improving the short-term neurological outcome.	Acute treatment of CVA with a single intravenous OGV dose provided strong neuroprotective effects and was most effective when administered immediately after the onset of ischemia. Thus, it proved to be a possible early treatment of ischemic stroke to prevent further brain damage.
Mayurasak orn et al. (2016).	United States	To test the hypothesis that the administration of docosahexaenoic acid (tri-DHA) enriches the brains that have suffered hypoxia-	10-day-old mice, after HI injury, received tri-DHA, tri-EPA or vehicle. The composition of mitochondrial fatty acids and the buffering capacity of Ca ²⁺ at 4-5 hours of reperfusion were analyzed; at 24 hours and at 8-9 weeks, oxidative damage, neurofunctional and neuropathological results were evaluated. In	Post-treatment with tri-DHA reduced oxidative damage and improved short- and long-term neurological outcomes, associated with increased DHA content in brain mitochondria and DHA-derived bioactive	The interaction of DHA with the mitochondria changes the release of ROS and improves the buffering capacity of Ca ²⁺ . This may explain the neuroprotective action of post-HI administration of tri-DHA.

		ischemia (HI) lesions with DHA / DHA metabolites, reducing the permeabilization of the mitochondrial membrane induced by Ca ²⁺ .	in vitro, mitochondrial ROS generation induced by hyperoxia and Ca ²⁺ buffering capacity were measured in the presence or absence of DHA or EPA.	metabolites in brain tissue. In vitro, hyperoxia increased the mitochondrial production of ROS and reduced the buffering capacity of Ca ²⁺ ; DHA has mitigated these effects.	
Shi et al. (2016)	China	To investigate the neuroprotective effects of combining omega-3 polyunsaturated fatty acids (n-3 PUFAs) with Lycium barbarum polysaccharide (LBP) in cortical neurons using an in vitro ischemic model.	Primary cultures of cortical neurons and oxygen-glucose deprivation/reperfusion (OGD/R), immunohistochemistry, genomic DNA extractions and PCR amplification, fatty acid analysis, cell viability assay, TUNEL staining, intracellular calcium measurements were performed (Ca ²⁺) and Western Blotting analysis were performed.	Treatment with docosahexaenoic acid (DHA) inhibited the increase of intracellular Ca ²⁺ in cultured WT cortical neurons submitted to OGD/R. Treatment with LBP activated Trk-B signaling in cortical neurons and attenuated cell apoptosis. Combining LBP with n-3 PUFAs in WT neurons showed effects on the protection of WT neurons against OGD/R injuries	It was pointed out that omega-3 polyunsaturated fatty acids and LBP are promising candidates for combined pharmacotherapy for ischemic stroke.
Nobre et al (2016)	Brazil	To study the neuroprotective effects of omega-3 (ω -3) in a model of global ischemia.	Male Wistar rats were submitted to carotid occlusion (30 min), followed by reperfusion. The groups were the false operated (FO), ischemic untreated (NT) with ω -3 and ischemic treated (ST) with ω -3 (5 and 10mg / kg for 7 days). The animals in the FO and NT groups were treated by oral with 1% cremophore and, 1 hour after the last administration, they were treated and tested behaviorally and sacrificed for neurochemical, histological analysis and immunohistochemical evaluations. The data were analyzed by ANOVA and Newman-Keuls.	Ischemia increased locomotor activity and breeding behavior which were partially reversed by \pm 3. Ischemia decreased the striated content of dopamine (DA) and DOPAC and increased the content of NE, effects reversed by ω -3. This medication protected the hippocampus from neuron degeneration and increased immunostaining for TNF-alpha, COX-2 and NOSs were partially or totally blocked by ω -3.	This study showed a neuroprotective effect of ω -3, largely due to its anti-inflammatory effect, stimulating translational studies focusing on its use in stroke management clinics.

Zhang et al (2016)	China; United States	To examine the effects of n-3 polyunsaturated fatty acids (n-3 PUFAs) on the integrity of the blood-brain barrier (BBB) after neonatal hypoxic-ischemic (HI) injury.	The rats were fed a diet with or without enrichment of AGPI3 from the second day of pregnancy until 14 days after delivery. H / I was introduced into the 7-day-old offspring.	AGPI3 reduced H / I-induced BBB damage, as shown by reductions in tracer efflux and IgG extravasation, preservation of BBB ultrastructure and increased expression of the narrow-junction protein. In addition, AGPI3 prevented increased matrix metalloproteinases (MMPS) activity in the brain and blood after H / I.	Thus, n-3 PUFAs can protect newborns against BBB damage by reducing the activation of matrix metalloproteinases after HI.
Saber et al. (2017)	United States	To investigate the relationship between circulating eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) with the risk of ischemic stroke, atherothrombotic and cardioembolic.	The levels of circulating phospholipid fatty acids (PLFAs) were verified at the beginning of the study in 3 cohorts. Ischemic strokes were classified as atherothrombotic or cardioembolic. The risk according to PLFA levels was assessed using Cox proportional hazards or conditional logistic regression according to the study design. Cohort findings were grouped using fixed-effect meta-analysis.	After multivariate adjustment, a lower risk of total ischemic CVA was observed with the highest levels of DPA and DHA, but not with those of EPA. DHA was associated with a lower risk of atherothrombotic stroke and DPA with a lower risk of cardioembolic stroke.	In 3 large US cohorts, the highest circulating levels of DHA were inversely associated with incident atherothrombotic stroke and APD with

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Discussion

In the post-stroke period, the metabolic demand of tissues increases considerably, leading the patient to lack several essential nutrients, such as omega-3 (Bourourou; Heurteaux & Blondeau, 2016). This malnutrition slows down and impairs the regeneration of brain tissue. To reverse this situation, studies have shown how long-chain omega-3 fatty acids (DHA and EPA) (Berressem; Koch; Franke, Klein & Eckert, 2016) and their alpha-linoleic acid precursor (ALA) (Bourourou et al., 2016) administered, respectively, by injections and by food supplementation have neuroprotective properties. As much as there are disagreements regarding these studies, such as the statement by Berressem et al., (2016) that there is a reduction in the infarcted area in the action of omega-3 and the statement by Bourourou et al.,

(2016) that the neuroprotective effect is due to the improvement in learning and memory and not to the reduction of the infarcted area, both confirm mechanisms by which omega-3 has the beneficial effects in the post-stroke period.

The administration of a single dose, in female rats, of a long-chain omega-3 lipid emulsion (Omegaven 10% ®, OGV) containing fish oil (DHA 18mg/ml; EPA21mg/ml) and alpha-tocopherol (0,2mg/ml) demonstrated great efficacy in the acute treatment of ischemic stroke. When this treatment is performed exclusively with EPA, it has been shown to be ineffective in the hypoxia-ischemia brain injury model, unlike DHA, which showed great efficacy in the same experiment; however, EPA demonstrated effects such as vasodilation and better fluidity of the membrane, which, in combination with DHA, it can enhance the neuroprotective effects (Berressem et al. 2016). Studies by Nobre et al., (2016) demonstrate that small doses (5 and 10 mg/kg/day, orally) of omega-3 fatty acids administered for seven days show neuroprotective action, showing reversal of biological changes resulting from cerebral ischemia, such as the presence of anxiety, loss of spatial memory, and decreased expression of pro-inflammatory cytokines in the studied nervous tissue.

Cerebral ischemia in neonates may have its consequences mitigated with the administration of omega-3 polyunsaturated fatty acids (n-3 PUFA) (Zhang et al, 2010; Zhang et al., 2015; Zhang et al, 2016), with various pathophysiological mechanisms for this interaction being described in the literature. Zhang et al., (2016) inferred that a n-3 PUFA enrichment diet, in the period from the second day of pregnancy to two weeks after delivery, acts in maintaining the integrity of the blood-brain barrier in Sprague-Dawley rats. This is due to the suppression of the activity and of the synthesis of matrix metalloproteinases, enzymes that play a role in the degradation of the basement membrane proteins and the stock junction, and that, when their production is suppressed, the levels of pro-inflammatory cytokines such as TNF-alpha and IL-1beta are also suppressed.

Zhang et al., (2015) also presents another mechanism by which n-3 PUFA acts in a neuroprotective manner, in which the dietary supplementation of n-3 PUFA enriched in female rats on the second day of gestation will considerably increase the content of this fatty acid in the cerebral cortex, suppressing, by n-3 PUFA, the inflammatory response, in addition to promoting phosphatidylserine biosynthesis in neuronal cell membranes. Phosphatidylserine acts as a protective factor for cell survival, as it preserves and increases a survival path called PI3K/Akt, which has been increasingly linked to neuronal survival in post-ischemic damage. Another study that reports the protective action of omega-3 in cerebral ischemia, in Zhang et al (2010), states that n-3 PUFAs show a decrease in their amount after CVA due to the increased metabolic demand. However, when omega-3 supplementation occurs, the neuroprotective effect occurs by partially suppressing the inflammatory response mediated by microglia.

In addition, Mayurasakorn et al. (2016), describes that mice that, at 10 days of age, experienced brain damage due to ischemia showed short (24 hours) and long term (8 to 9 weeks) improvement in the neurological progress after treatment with two administrations via intraperitoneal doses (0.375 g n-3 TG/kg/dose) of tri-DHA, and not of tri-EPA. One administration was performed immediately after the ischemic damage and the other after 1 hour. Such improvement was associated with an increase in DHA in brain mitochondria and bioactive metabolites derived from DHA in brain tissue. This fact was associated with mitochondria that suffered hypoxia-ischemia becoming resistant to calcium-induced membrane permeability (Ca²⁺), reduced oxidative brain damage and permanent neuroprotection. This corroborates the studies by Zhang et al., (2010); Zhang et al., (2015); Zhang et al., (2016).

The presence of a low proportion of n-3 polyunsaturated fatty acids (n-3 PUFAs) may be associated with ischemic and hemorrhagic damage, in cerebral small vessel diseases (CSVD), including the total score, which measures the severity of the disease. Thus, a diet rich in n-3 PUFA, could reduce the severity of CSVDs, regardless of their hemorrhagic or ischemic origin (Song et al., 2015).

In a population-based study in South Korea with 220 patients, high levels of n-3 PUFAs in the blood were associated with better executive functions and beneficial effects on the white matter microstructure, while low levels of n-3 PUFAs were related to more serious changes in white matter. One of the protection mechanisms would be the production of anti-inflammatory molecules, resolvins and protectins, through the lipoxygenase or cyclooxygenase pathways. In addition, n-3 PUFA had an effect on the stabilization of atheromatous plaques and lesser macrophage infiltration in patients waiting for a carotid endarterectomy who were treated with fish oil (Song et al., 2015). In contrast, in 3 independent prospective cohorts, which included different samples of men aged 40 to 65 and women aged 30 to 55, performed in the United States, it was found that not all active forms of n-3 PUFA, acid eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), have the same effectiveness in reducing risk of total ischemia, atherothrombotic and cardioembolic CVA (Saber et al., 2017).

Thus, among the types of ischemic infarction, circulating DHA is inversely associated with atherothrombotic stroke, whereas DPA, with cardioembolic stroke. However, EPA was not associated with a lower risk of ischemic CVA, total atherothrombotic or cardioembolic. It was found that higher levels of DHA represented 47% lower risk when assessing atherothrombotic stroke, without significant relevance of circulating DPA and EPA in these cases. When considering cardioembolic stroke, patients with the highest levels of DPA, compared to the lowest levels, had 42% lower risk, without significant relevance of the amounts of blood DHA and EPA in such cases (Saber et al., 2017).

DHA is also a precursor to neuroprotectin D1, a docosanoid anti-inflammatory derivative which reduces apoptosis, promoting cell survival in ischemic stroke models (Marcheselli et al., 2003).

Furthermore, Lin et al., (2015) suggests that DHA would also act in the hemorrhagic transformation (HT), a feared complication of ischemic stroke, frequent after the use of thrombolytic therapy. In this sense, DHA would act in order to reduce the risk of HT, which is increased due to hyperglycemia after focal ischemic injury. The studies by Lin et al., (2015) used adult male rats that received 50% dextrose injection (6 ml/kg intraperitoneal) in order to induce hyperglycemia and, consequently, HT, 10 min before suffering a middle cerebral artery occlusion. Treatment with DHA (10mg/kg) 5min before reperfusion reduced HT, decreased the volume of infarction, in addition to having demonstrated better neurological progress after seven days. It was also confirmed that DHA inhibited the inflammatory reaction mediated by ICAM-1, a transmembrane protein in endothelial cells that facilitates leukocyte transmigration and adhesion. DHA also acted by inhibiting the collagen type IV degradation, in order to stabilize the blood-brain barrier, since collagen IV is the main component of capillary endothelial cells.

Still under this bias, Sumiyoshi et al., (2015) evaluated that in ovariectomized female rats that were subjected, after the eleventh week of life, to cerebral ischemia due to middle cerebral artery occlusion, there is an increase in the expression of HMG-1, a DNA-binding ubiquitous nuclear protein that is released by necrotic cells and secreted by activated leukocytes. Such protein has relevance in post-ischemia damage, acting as an activator of antigen-presenting cells through the receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs), triggering and exacerbating the inflammatory response. It has also been shown that a pretreatment with EPA supplementation (500 mg/kg/day, orally) for 4 weeks attenuates these changes regardless of estrogen levels, which may point to a future treatment in cases of human women affected by stroke and who are in the post-menopausal stage. EPA, in addition to having antiarrhythmic, anticoagulant, antioxidant, and anti-inflammatory actions, is also an agonist of the PPAR γ receptor, which modulates the immune system of the central nervous system by inhibiting the activation of macrophages and by modulating the release of pro-inflammatory mediators (Sumiyoshi et al., 2015).

In a study with an animal model of oxygen-glucose ischemia/reperfusion injury or oxygen-glucose deprivation/reperfusion (OGD / R), fat-1 type rats, that is, those with a higher level of endogenous n-3 PUFA, were fed with 10% corn oil. Shi et al., (2015), then, demonstrated that both endogenous and exogenous n-3 PUFA increased the survival of a culture of cortical neurons after OGD / R. In this model, the fat-1 gene increased the expression of neuronal n-3 PUFA, including the active forms DPA and DHA. Thus, when comparing fat-1 rats with wild-type rats (WT), it was found that WT neurons showed cellular

damage and neuritis 24h after OGD/R considerably greater than fat-1 neurons. In addition, lower intracellular accumulation of reactive oxygen species (ROS) in fat-1 neurons was attested, which points to inhibition of ROS activation by endogenous n-3 PUFA. There was also greater expression of cleaved caspase-3 in WT neurons. In the exogenous administration of DHA and GSH, there was also a significant reduction in ROS activation. Such neuroprotection is directly related to the elimination of ROS and positive regulation of anti-apoptotic proteins. Recently, it was discovered that DHA modulates neuronal defenses by activating the expression of GSH in brain cells. The observations also showed a significant improvement in the integration of motor and sensory functions, 2 weeks after OGD / R, in fat-1 rats.

Shi et al. (2016) added that, by combining n-3 PUFA treatment, especially DHA, with *Lycium barbarum* Polysaccharide (LBP), better results can be obtained in the rescue of cortical neurons after OGD/R, particularly by activating the TrkB receptor and alleviating intracellular Ca²⁺ overload. In this context, studies have pointed out the role of n-3 PUFA in inhibiting the release of Ca²⁺ by the endoplasmic reticulum. However, in this research, treatment with only LBP proved to have limited influence on the control of Ca²⁺ overload. Furthermore, the observations demonstrated that LBP and n-3 PUFA perform neuroprotection by activating the anti-apoptotic Bcl-2 cascade, a fact that also contributes to maintaining Ca²⁺ homeostasis in the endoplasmic reticulum. This study, for the first time, also reported that LBP can perform neuronal protection from apoptosis by modulating the neurotrophin pathway, which is initiated from the cell membrane. However, contrary to Shi et al., (2015), it was stated that there are inconsistencies in the dietary supplementation of n-3 PUFA in animal studies due to the variance of the component in the diet, in addition to a certain neglect of the relevance in the proportion between n-3 PUFA and n-6 PUFA (Shi et al., 2016).

Conclusion

Omega-3 can possibly act as a neuroprotective agent in several ways, playing an important supporting role in the prevention and/or treatment of CVA. Studies have shown its vasodilator activity, providing improvement in spatial memory and learning and decreased depression and anxiety after global ischemia in animals.

Its anti-inflammatory potential has also been reported, decreasing the activation of microglia and release of pro-inflammatory mediators, restoring mitochondrial function and reestablishing glucose level in brain tissue. Studies also report that omega-3 can act to reduce cerebral edema, promote angiogenesis, preserve blood-brain barrier integrity and protect

tissue by reducing reactive oxygen species. This study was limited due to the existence of few records in the literature that address the direct relationship between protective properties of omega-3 and ischemic stroke, in addition to sparse research that includes human models. Thus, there is a need for further research in the area, in order to provide greater understanding about the molecular action of omega-3 fatty acids and their role in supplementation in humans.

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