Chapter

Aneurysmal Subarachnoid Hemorrhage and Resolution of Inflammation

Geisi Saito and Rodrigo Zapata

Abstract

Aneurysmal subarachnoid hemorrhage (SAH) is a severe life-threatening disease and an important source of neurological disability. Therapeutic interventions over the last few decades have repeatedly failed to improve functional outcome after SAH; however, resolution of inflammation has largely been ignored as a potential therapeutic target. The omega-3 fatty acids (FAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the precursors of key mediators involved in resolution of inflammation and endogenous neuroprotection. EPA also plays a major role in microvascular function, and DHA accretion in the brain is crucial for normal neuronal function. Although considerable loss of brain DHA has been identified in SAH patients, the pathological significance of this process has also been overlooked. Current Western diets provide insufficient amounts of omega-3 FAs to compensate for the loss of brain DHA following SAH. Here, we review the rationale for future clinical trials of omega-3 FAs in SAH. Furthermore, the potential role of defective resolution of inflammation in the growth and rupture of intracranial aneurysms is inferred from recent findings in atherosclerosis and nutrition. The novel concepts of resolution of inflammation and endogenous neuroprotective signaling may open new avenues for public health interventions and innovative research in intracranial aneurysms and SAH.

Keywords: fish oil, inflammation, intracranial aneurysms, omega-3 fatty acids, pharmaconutrition, subarachnoid hemorrhage, translational approach

1. Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is a complex condition with an intricate and poorly understood pathophysiology. Increasing evidence strongly suggests that neuroinflammation plays a critical role after SAH, but conventional anti-inflammatory treatments have failed to improve clinical outcome [1]. Clinical research on SAH has mainly focused on delayed cerebral ischemia (DCI); however, DCI does not encompass the entire spectrum of pathological and clinical manifestations of SAH [2–4]. Although cerebral infarction is associated with poor clinical outcome and death after SAH, a significant proportion of SAH patients without cerebral infarction suffer from cognitive deficits and mood disorders and a reduced ability for activities of daily living and working, even in the long term [5, 6]. The absence of a close correlation between DCI and functional recovery indicates an

ongoing pathophysiological process has been overlooked in SAH. The failure of the recent major NEWTON 2 clinical trial, of sustained intraventricular release of nimodipine, is the latest in a series of unsuccessful phase 3 randomized controlled trials (RCTs) to improve clinical outcome after SAH and further reinforces the need to identify novel therapeutic strategies [7, 8].

Nutrition is essential to human health, and appropriate nutritional support is currently considered a standard of care for critically ill patients. Malnutrition—including depletion of essential micronutrients—frequently occurs among critically ill patients and is associated with an increased risk of morbimortality [9]. However, the clinical relevance of key nutrient deficiencies in acute neurological illnesses has not been thoroughly investigated. EPA and DHA are essential constituents of endothelial and neuronal membranes, respectively, and also the precursors of key mediators involved in resolution of inflammation and endogenous neuroprotection [10, 11]. Although massive loss of brain DHA in SAH patients was first reported over 15 years ago, the pathological significance of this process and the role of inflammation resolution following SAH have largely been ignored [11, 12]. Therapeutic interventions aimed at stimulating inflammation resolution and recovering the homeostasis of the brain and other vital organs after SAH could improve patients' functional outcome [13].

This chapter provides an overview of the potentially harmful consequences of selective deficiency of omega-3 FAs on brain structure and function in SAH patients. Moreover, given the possible clinical relevance to SAH and the growth and rupture of intracranial aneurysms (IAs), we provide a detailed discussion of recent findings on the role of omega-3 FAs in resolution of inflammation, with a focus on brain homeostasis.

2. Role of omega-3 FAs in resolution of acute inflammation

Remarkable progress in our understanding of the pathophysiology of acute inflammation has been achieved through basic science over the last 20 years. Resolution of acute inflammation is now considered to be an active biochemical process that is required to enable tissues to restore normal structure and function following an injury [14]. Interference with the resolution phase of acute inflammation may result in necrosis, chronic inflammation, fibrosis, and organ dysfunction. The resolution process is triggered at the beginning of inflammation by a temporal switch in lipid mediator classes, which is induced by cross talk between cells of the innate immune system and other cells in the inflammatory microenvironment [11].

A diverse range of biologically active pro-resolving and anti-inflammatory mediators are synthetized by complex pathways that involve several enzymes, including cyclooxygenase 2 (COX-2), cytochrome P450s and several lipoxygenases (LOX). The majority of these endogenous mediators are derived from the long-chain omega-3 fatty acids (FAs) EPA and DHA and are members of the specialized pro-resolving mediator (SPM) superfamily [15]. SPMs represent an essential biochemical interface between inflammation and tissue repair and regeneration. The resolution of inflammation is a highly orchestrated adaptive process that depends on both the availability of SPMs precursors and the efficiency of the related biosynthetic pathways.

2.1 Specialized pro-resolving mediators

Each endogenous lipid mediator is structurally distinct and possesses specific biological functions which have been extensively studied in diverse experimental

models. EPA is the precursor of the E-series resolvins (RvEs), which contains four main mediators (RvE1-RvE3 and 18-HEPE). DHA is the precursor of the D-series resolvins (RvDs), which contains six mediators (RvD1-RvD6), as well as the protectins (PD1-PDX) and the maresins (MaR1-MaR2); DHA derivatives are also known as docosanoids [15].

SPMs are active at pico-nanogram ranges and exert pleiotropic actions at the inflammatory microenvironment. SPMs stimulate the clearance of bacteria, dead cells, and debris that is required during tissue repair. SPMs also reduce leukocyte transendothelial migration, platelet activation, and production of inflammatory cytokines, thus providing multi-organ protection. The maresins (macrophage mediators in resolving inflammation) exert potent phagocyte-directed actions that include phenotypic conversion of proinflammatory macrophages into macrophages that suppress inflammation and promote tissue regeneration [16]. Collectively, SPMs actively promote resolution of inflammation and recovery of tissue homeostasis [15]. Interestingly, resolution of systemic inflammation appears to have its counterpart in the CNS represented by different DHA-derived endogenous mediators (see Section 5.1).

The biosynthetic pathways that generate SPMs are clinically relevant and have been comprehensively studied [15, 16]. Drugs that inactivate the enzymes involved in SPM biosynthesis, such as selective COX-2 inhibitors and certain LOX inhibitors, delay the return to homeostasis and are considered resolution antagonists. Importantly, selective COX-2 inhibitors were synthesized before the identification of inflammation resolution pathways and are currently widely used as anti-inflammatory agents. Aspirin and statins also modify COX-2 by acetylation and S-nitrosylation, respectively, which results in generation of longer-acting SPM R-epimers. Thus, aspirin and statins are resolution agonists [15, 16].

3. Incorporation and transport of omega-3 FAs

Incorporation and transport of omega-3 FAs have been comprehensively described; here, we focus on the clinically relevant aspects [10, 17, 18]. Several studies have consistently shown that in vivo conversion of alpha linolenic acid (ALA), the short-chain omega-3 FAs from vegetable origin, to its bioactive long-chain derivatives (EPA and DHA) is very low in humans. In addition, the metabolism of omega-6 and omega-3 FAs is tightly linked, and thus a high dietary intake of omega-6 FAs further reduce the conversion of ALA to EPA and DHA and their biological effects. The body has also a limited capacity to store long-chain omega-3 FAs; only very small amounts of EPA and DHA are present in adipose tissue, and the brain retains DHA for its own function. Thus, providing preformed EPA and DHA is the most efficient method of increasing the concentrations of EPA and DHA in plasma and tissues.

EPA and DHA are incorporated into different blood lipid fractions after absorption by the gastrointestinal tract or after release from intravenously infused fish oil-based lipid emulsions (FOLE). These lipid fractions reflect the diverse means by which FAs are transported in the circulation and execute their physiological functions. The fractions incorporated in the phospholipid coat of plasma lipoproteins and plasma nonesterified FAs (NEFAs) are considered transport pools. Notably, the NEFA pool seems to be the main DHA plasma fraction that supplies the brain. The NEFA pool also represents a direct source of FAs to cells for generation of SPMs, as this pool readily transfers to inflammatory tissue via edema formation [19]. The FAs fraction incorporated in peripheral blood mononuclear cells (PBMCs) represents a functional pool due to the crucial roles of PBMCs in inflammation.

3.1 Omega-3 index

The EPA and DHA content of red blood cells (RBCs) membrane which can be quantified by a specific and standardized analytical procedure—the HS-Omega-3 Index® methodology—reliably reflects the omega-3 FAs content of several tissues [20]. This omega-3 index has been increasingly utilized as a surrogate marker of omega-3 status. Long-term intake of EPA and DHA is the main predictor of the omega-3 index, but other factors influence its variability. Acute supplementation of omega-3 FAs does not modify the omega-3 index, as expected given the long lifetime of RBCs (100–120 days). In terms of clinical relevance, strong inverse correlations have been observed between the omega-3 index and cardiovascular morbimortality, particularly sudden cardiac death, as well as depression [21–23].

4. Role of EPA in microvascular thromboinflammation

Microvascular inflammation is an early event in the pathogenesis of atherosclerosis and other inflammatory conditions and is inextricably linked to microthrombosis [24]. Eicosanoid metabolism and leukocyte-endothelial interactions are interrelated processes and in turn are major drivers of thromboinflammation [25, 26]. Inflammation stimulates eicosanoid synthesis and expression of cell-surface adhesion molecules through upregulation of the nuclear factor kappa B (NF-kB), a master transcription factor necessarily involved in the inflammatory response.

Most eicosanoids derived from the long-chain omega-6 FAs arachidonic acid (ARA), including prostaglandins, leukotrienes, and thromboxanes, are potent vasoconstrictors, platelet activators, and leukocyte chemotactic factors. Moreover, the expression of cell-surface adhesion molecules on endothelial and inflammatory cells is essential for leukocyte-endothelial interactions; rolling and adhesion on vascular surfaces are the first step in the recruitment of circulating leukocytes or platelets to sites of thromboinflammation [27].

EPA incorporated in the membrane phospholipids of inflammatory cells can modulate eicosanoid metabolism by replacing ARA as an eicosanoid precursor [25]. EPA-derived eicosanoids are significantly less potent than those derived from ARA, and nonesterified EPA can also directly inhibit the production of eicosanoids from ARA. In addition, EPA has been shown to decrease the expression of several cell-surface adhesion molecules on inflammatory cells. EPA appears to elicit these pleiotropic effects by modulating the activity of the NF-kB.

Additionally, recent mechanistic studies have shown that minor changes in the EPA content of endothelial membranes may markedly alter the biophysical properties of the membrane [28]. Furthermore, changes in membrane fluidity, thickness, and deformability induced by modifications to lipid dynamics and/or structural organization can profoundly impact endothelial function [29]. Given the intimate association between brain capillary pericytes and endothelial cells, it would not be surprising if EPA also incorporates into pericyte cell membranes and potentiates the function of these cells as regulators of brain homeostasis [30, 31].

5. Unique function of DHA in the brain

DHA is widely distributed throughout the human body in membrane phospholipids and is particularly abundant in neural tissues such as the brain and retina [10]. DHA represents 30–40% of the fatty acid content of the gray matter in the cortex and is absorbed into the brain by a specific transporter that is found in

the endothelium of the blood-brain barrier (BBB) microvasculature [32, 33]. The functional significance of selective enrichment of DHA in neural tissues has been actively researched over the last few decades. DHA possesses unique biophysical and biochemical characteristics that make it particularly suitable for brain and retinal membranes. DHA has been highly conserved during evolution and is present throughout the entire spectrum of living organisms [34]. DHA has even been suggested to be a major determinant for evolution of the modern hominid brain due to its unique encephalization potential [35].

DHA is an essential component of neuronal membrane architecture and composition and promotes selective accumulation of phosphatidylserine (PS), a crucial phospholipid involved in intracellular signaling [36]. PS participates in the signaling events of several key enzymes, including protein kinase C, Raf-1 kinase, and Akt, which play essential roles in cell proliferation, differentiation, and survival. Thus, DHA significantly modulates the activity of essential cellular kinases in neuronal cells.

5.1 DHA and endogenous neuroprotective signaling

In addition to its function as a unique building block of cell membranes, DHA is also a precursor for docosanoids and other bioactive endogenous derivatives in the neural tissue [37]. The number of recently identified DHA derivatives in neural tissue is increasingly growing and includes neuroprotectin D1 (NPD1), synaptamide, endocannabinoid epoxides, and elovanoids [38–40]. Collectively, the potent bioactive properties of these DHA derivatives contribute to preservation of normal neuronal function, tissue homeostasis, and neuronal survival [37–41]. In addition, the DHA derivatives exert a range of potent neuroprotective properties that include inhibition of proinflammatory gene expression and leukocyte infiltration. A striking hallmark of the DHA derivatives is their ability to potentiate microglial polarization from a proinflammatory phenotype to a surveillance-repair state and reduce NF-kB-mediated expression of inflammatory cytokines in the brain. Moreover, DHA derivatives contribute to BBB integrity and are neurogenic and synaptogenic [38, 42]. Thus, the DHA derivatives seem to be key mediators of the resolution of inflammation and recovery of homeostasis in the CNS microenvironment.

5.2 DHA and neurovascular unit

The concept of neurovascular unit emphasizes the intimate relationship between the brain and its vessels, particularly the coupling between neural activity and cerebral blood flow [43]. Although the role of DHA neurolipidomics in neurovascular coupling appears to be underestimated, substantial experimental evidence suggests that the morphologic and functional integrity of the neurovascular unit largely depends on high DHA enrichment [37, 40, 43]. Moreover, the potential role of EPA in microvascular function further supports the evolutionary importance of these essential nutrients to maintain efficient functional couplings between neural and vascular networks [28, 29]. A functional neurovascular unit may be crucial not only for neurovascular coupling but also for BBB integrity and neurogenesis.

A regular dietary supply of DHA is required to preserve normal brain and retinal function. Under physiologic conditions, the net DHA incorporation rate for the entire human brain is very low and equivalent to the net rate of DHA consumption by the brain (3.8 ± 1.7 mg/day) [44]. However, loss of DHA in pathological states or due to nutritional deficiency of omega-3 FAs may severely impair neurovascular integrity and have far-reaching implications on normal brain function [36].

6. Loss of brain DHA after SAH

To date, three clinical studies have examined the loss of DHA from the brain after SAH. Pilitsis et al. conducted the first observational study to analyze free fatty acid (FFA) concentrations in cerebrospinal fluid (CSF) [12]. The concentrations of ARA, DHA, linoleic acid, myristic acid, oleic acid, and palmitic acid were measured over the first 14 days following SAH in 20 patients. A cohort of 73 patients with no evidence of acute neurological disease served as the control group. Compared to control patients, the concentrations of all FFAs tested were significantly elevated in CSF during the first 2 days after SAH, with a significant secondary increase in FAA concentrations at 8–10 days. The concentrations of DHA exhibited a biphasic increase and remained significantly elevated (200–600%) throughout the first 14 days after SAH.

Increased levels of free DHA in CSF after SAH are likely to be the result of the cleavage of DHA from membrane phospholipids by either direct structural damage or an increase in phospholipase A2 activity in response to neuroinflammation. DHA can also be readily oxidized due to its high degree of unsaturation and excessive generation of free radicals following SAH [45]. Such nonenzymatic oxidation of DHA generates F4-neuroprostanes (F4-NPs), which represent a lipid marker of oxidative stress in the CNS. Two clinical studies confirmed that the concentrations of F4-NPs in CSF significantly increased within the first 24 hours following SAH compared to control patients [46, 47]. Hsieh et al. also showed the concentrations of F4-NPs in CSF remained significantly elevated throughout the first 10 days after SAH and suggested a positive correlation exists between F4-NP concentrations and clinical outcome at 3 months after SAH. Despite the limited number of patients analyzed, these studies provided valuable evidence that substantial loss of brain DHA occurs after SAH.

Moreover, it is highly likely that SAH may increase metabolic consumption of DHA though increased generation of neuroprotective derivatives. This potential additional source of DHA loss has not yet been evaluated but could be significant. Net cumulative DHA loss from the brain (DHA loss + DHA consumption) following SAH may be massive in some cases and is likely to impose a severe burden on the brain.

6.1 Selective brain malnutrition

A current Western diet may provide sufficient amounts of FAs to compensate for the loss of other FAs, but not DHA. Current Western diets are characterized by very low intakes of long-chain omega-3 FAs and high intakes of other FAs, especially omega-6 FAs such as ARA and linoleic acid [48]. Thus, a significant imbalance between brain DHA loss and inadequate nutritional intake of omega-3 FAs may persist over the long term in SAH patients, hindering the recovery of DHA accretion in the brain required for normal neuronal function [35, 36].

Loss of EPA after SAH has not yet been examined; however, it is reasonable to assume that depletion of EPA from cerebral endothelial membranes may play a significant role in microvascular dysfunction after SAH. Indeed, EPA seems to have a more potent effect than DHA in the treatment of mood disorders, though the underlying mechanisms remain elusive [22].

We coined the term “selective brain malnutrition” to describe the pathological consequences of EPA and DHA loss following SAH on the structure and function of the brain. This novel hypothesis of selective brain malnutrition offers a plausible explanation for some of the intriguing clinical features of SAH, including diffuse cerebral atrophy and the frequently observed long-lasting functional sequelae, such

as cognitive dysfunction and mood disorders, that occur even in the absence of focal injury [5, 49]. Importantly, a higher omega-3 index has been associated with larger total brain and hippocampal volumes in observational studies in humans [50].

7. Roles of EPA and DHA in resolution of inflammation after SAH

Consensus has emerged on the pressing need to find a multipronged therapeutic intervention to address the various deleterious effects of early brain injury (EBI) after SAH [51]. Nonetheless, the loss of brain DHA after SAH is likely to be an unrecognized effect of EBI, and in turn loss of DHA may represent a critical event in the pathogenesis of secondary brain injury after SAH. Depending on the severity of SAH, the cumulative burden of brain DHA loss may be massive and decreases endogenous neuroprotective capacity in the short term [12, 36]. Thus, unresolved homeostatic disturbances within the cerebral microenvironment may lead to neurovascular uncoupling, which may spread over the cerebral cortex in the most severe cases [52]. The loss of an entire series of signaling events required for maintenance of neurovascular network integrity may further increase the risk of focal injury, diffuse cerebral atrophy, and functional sequelae [37]. In this context, it is reasonable to assume that large-artery vasospasm may paradoxically be a compensatory mechanism to preserve tissue oxygen availability in the presence of progressive microvascular failure, i.e., when capillary transit time heterogeneity substantially increases [3, 31]. Unresolved inflammation may also induce hyperproliferation of arachnoid cap cells, which increases the risk of hydrocephalus [4]. Uncontrolled systemic complications, such as severe cardiopulmonary dysfunction, may further aggravate homeostatic disturbances and have devastating consequences on brain function [53].

Theoretically, EPA, DHA, and their respective SPMs possess the bioactive capacity to counteract the major homeostatic disturbances that occur after SAH. EPA-RvEs could reduce thromboinflammation at the cerebral microvasculature by inhibiting vasoconstriction, leukocyte transendothelial migration, and platelet aggregation [15, 26]. DHA and its derivatives may trigger the critical signals required to maintain functional neurovascular coupling and cell survival [16, 36, 37]. DHA-induced upregulation of the enzyme heme oxygenase 1 (HO-1) may accelerate the clearance of subarachnoid clots and thus decrease heme-induced cerebral inflammation [54]. SMPs may attenuate inflammation-induced hyperproliferation of arachnoid cap cells, further contributing to diminish the risk of hydrocephalus. SPMs may also provide multi-organ protection and enhance the immune response against infections.

Furthermore, the promising role of DHA derivatives in reducing microglial polarization toward an inflammatory phenotype may offer a novel approach to reduce the brain inflammation induced by neurosurgical trauma in surgically treated SAH patients [55]. Neurogenesis has also been identified in SAH patients, and thus DHA could represent a novel therapeutic strategy to improve neurological recovery by stimulating neurogenesis [56]. Moreover, subtle changes on microvascular function and synaptogenesis induced by EPA and DHA may improve cognitive function and mood and thus increase the likelihood of complete functional recovery of SAH patients.

7.1 Clinical rationale for omega-3 FAs therapy in SAH

The theoretical framework described above provides scientific rationale for future clinical trials of omega-3 FAs in SAH patients. The disappointing results

of RCTs of isolated pharmaceutical interventions in SAH could be overcome if a translational approach is correctly implemented [8]. In support of this notion, the recently published phase 3 REDUCE-IT trial provided a robust demonstration of the clinical efficacy and therapeutic potential of EPA to reduce cardiovascular events in chronic cardiovascular (CV) patients with hypertriglyceridemia [57]. Obviously, there are major differences between patients with chronic CV disease and hypertriglyceridemia and patients with SAH. Nevertheless, the considerable clinical benefits of EPA observed in patients with CV disease (who were already on statin therapy) may be due not only to the triglyceride-lowering effect of EPA. Interestingly, the results of the REDUCE-IT trial resemble those of the GISSI-Prevenzione trial conducted 20 years ago, in which a low-dose oral treatment of EPA plus DHA significantly reduced CV morbimortality in patients who had suffered a recent myocardial infarction and were already on aspirin treatment [58].

Minor changes in overall membrane FAs composition and the increase in local production of longer-acting SPM R-epimers after combined treatment with omega-3 FAs and aspirin or statins are likely to mediate a wide variety of biological effects and have a profound impact on cellular and multi-organ function [15]. Indeed, emerging evidence clearly indicates a strong association between a higher omega-3 index and major health benefits (reduced risk of both CV and all-cause mortality) over the long term [21]. However, the acute and critical nature of SAH imposes a particularly demanding therapeutic challenge as the homeostatic disturbances in the brain and other vital organs must be effectively addressed in the short term. An intervention based exclusively on oral supplementation with omega-3 FAs is unlikely to be fully effective on its own in the clinical context of SAH. However, the superiority of parenteral administration over oral or enteral administration of omega-3 FAs has been reliably demonstrated in short-term interventions [17, 59]. Parenteral administration of omega-3 FAs allows rapid delivery of higher doses of EPA and DHA without bioavailability issues and does not depend on the patient's clinical condition. Indeed, the incorporation profiles of EPA and DHA in the transport and functional pools after a single parenteral dose are equivalent to up to several weeks of oral supplementation. EPA and DHA appear in PBMCs as rapidly as 6 hours after a single parenteral dose, thus highlighting the ability of parenterally administered omega-3 FAs to easily reach the innate immune system [59]. The concentration of omega-3 FAs in the main plasmatic fraction that supplies the brain and other vital organs (the NEFA pool) also increases rapidly after a single parenteral dose [17, 60]. Thus, the SPM precursors EPA and DHA seem to be rapidly and widely available to activate resolution of inflammation on demand in different organs after parenteral administration. Therefore, parenteral administration may be the most efficient way to deliver omega-3 FAs during the acute stage of SAH. This novel therapy is in accordance with the concept of pharmaconutrition, in which key nutrients are utilized as pharmacological agents and delivered in appropriate doses via the most efficient administration route [61]. Oral or enteral administration may be suitable for medium- or long-term treatment when the patient's gastrointestinal function has completely recovered.

8. Omega-3 FAs dosage for clinical use

A regular supplementation dose for healthy subjects defined by several health associations worldwide is around 500–750 mg/day of EPA plus DHA and can be achieved by consuming a regular diet that contains two portions of fatty fish per week [62]. Therapeutic anti-inflammatory doses of EPA plus DHA are usually considered to be above 2 g/day [25]. A daily dose of at least 1 g EPA plus DHA/day (EPA > 60% DHA) has been shown to be effective in patients with depressive

disorders [22]. EPA doses above 1.8 g/day seem to be required to produce clinically meaningful effects on endothelial and vascular function [63]. In patients with age-related cognitive decline, 900 mg DHA/day improved learning and memory function [64]. Importantly, omega-3 FAs doses can be significantly reduced by decreasing the dietary intake of omega-6 FAs. This fact likely explains the notably different dosages of EPA required to obtain beneficial outcomes in patients with chronic CV disease in a Japanese trial (1.8 g/day) and the REDUCE-IT trial (4 g/day) conducted in 11 Western countries [48, 57, 63].

The therapeutic dose of parenteral fish oil (FO) to complement total parenteral nutrition (TPN) is of 0.1–0.2 g of FO/kg/day [59]. This dosage is equivalent to 1–2 ml/kg/day of a specific FOLE that contains 10 g of FO/100 ml.

8.1 Bioavailability of omega-3 FAs oral formulations

Bioavailability refers to both the speed of absorption and the quantity of the substance absorbed in the gastrointestinal tract. The bioavailability of omega-3 FAs oral formulations should be carefully considered as it may have direct clinical implications [65]. The bioavailability of EPA and DHA depends on the chemical form in which they are bound (phospholipids > triglycerides > free fatty acids > ethyl esters) as well as their Galenic form (i.e., microencapsulation, emulsification) and also matrix effects (capsule ingestion with concomitant content of food, fat content in food). Galenic form and matrix effects are the most important factors that influence the bioavailability of EPA and DHA. Thus, administration of high-quality pharmaceutical formulations with fatty meals is necessary to ensure the effectiveness of oral therapy.

9. Experimental models and clinical interventional studies of omega-3 FAs in SAH

9.1 Experimental models

To date, only two preclinical studies of omega-3 FAs in experimental models of SAH have been published. Yin et al. suggested that pre-treatment with omega-3 FAs by oral gavage elicited anti-inflammatory and anti-apoptotic effects in a rat model of SAH [66]. Zhang et al. showed intravenous administration of DHA may prevent oxidative stress-induced apoptosis by improving mitochondrial dynamics in a rat model of SAH induced by endovascular perforation [67]. However, the scarcity of preclinical studies on omega-3 FAs in SAH contrasts with the large number of experimental studies on ischemic stroke. The effects of omega-3 FAs (or specific derivatives) in neural tissue have been widely examined in experimental ischemia-reperfusion models [55, 68, 69]. These studies have consistently shown that omega-3 FAs significantly reduce cerebral infarction volume by around 40–50% and are associated with a drastic decrease in the neuroinflammatory response [70, 71]. Interestingly, the long-term neurobehavioral recovery in experimental models of ischemic stroke is associated with neuroprotective effects of DHA on both gray and white matter [55]. It is noteworthy that one of these studies used a specific FOLE that is widely approved for clinical use [70].

9.2 Clinical studies

A limited number of omega-3 FAs interventional studies have been performed in SAH patients [13, 72–74]. The main characteristics and findings of these studies

are summarized in **Table 1**. Two studies utilized EPA and DHA, and only one study included a parenteral regimen. In total, 229 patients with SAH have received an omega-3 FAs intervention; most patients were surgically treated (n = 223). Although two studies were published as RCTs, one study had significant methodological shortcomings in the randomization process that conferred a high risk of selection bias [74]. While these preliminary clinical studies reported encouraging results (see **Table 1**), high-quality RCTs are needed to confirm the benefits of omega-3 FAs in SAH patients.

9.3 Safety considerations

Evidence obtained over more than two decades in other clinical fields indicates omega-3 FAs interventions are unlikely to lead to serious clinical harm in SAH patients [25, 59]. Nevertheless, parenteral administration of omega-3 FAs may raise some safety concerns. Total parenteral nutrition is associated with an increased risk of complications in critically ill patients [9]. Furthermore, administration of lipid emulsions (LEs) may cause fat overload syndrome; the amount of fat administered and LE infusion rate are the primary risk factors. In this regard, it should be emphasized that the therapeutic dose of FO (0.1–0.2 g of FO/kg/day) is about one order of magnitude lower than that of regular LE (0.7–1.5 g of fat/kg/day) [59]. Additionally, the plasma clearance rate is faster for FAs administered in FOLE than soybean oil-based LEs. These unique features contribute to the good safety profile of FOLE. Fish oil has been widely used as a component of total parenteral nutrition and is associated with reduced rates of infection, shorter hospital stay, and decreased mortality, particularly in surgically treated patients [59].

Furthermore, isolated parenteral administration of FO has increasingly been used in pediatric patients with parenteral nutrition-associated liver disease (PNALD). Several case series published since 2006 have reported parenteral FO monotherapy (PFOM) remarkably improved clinical outcome of patients with PNALD [75]. Importantly, PFOM has demonstrated a good safety profile in these

Reference	Type of study	Population, n	Intervention	Main result
Yoneda et al. [73]	Prospective, non-randomized	n = 101 EPA = 73	Oral EPA: 1800 mg/day × 10 postoperative (PO) days	Reduction in vasospasm and cerebral infarction
Yoneda et al. [74]	RCT	n = 162 EPA = 81	Oral EPA: 2700 mg/day × 30 PO days	Reduction in vasospasm and cerebral infarction
Nakagawa et al. [72]	Retrospective study	n = 100 EPA + DHA = 55	Oral EPA: 1860 mg/day + oral DHA: 750 mg/day × 90 PO days	Reduction in vasospasm and cerebral infarction
Saito et al. [13]	Pilot RCT	n = 41 EPA + DHA = 20	Parenteral perioperative: 5 days Oral EPA: 1840 mg/day + oral DHA: 1520 mg/day × 8 weeks	No postoperative intracranial bleeding complications Easy-to-implement intervention

Table 1.
Summary of the features and outcomes of clinical interventional studies in SAH patients.

critically ill patients, even at FO doses up to 1.5 g FO/kg/day and overextended treatments beyond 4 weeks, well beyond the manufacturer's recommendations.

The aim of replacement FO therapy in PNALD is obviously different to SAH patients. Parenteral administration of FO is intended to address key nutrient deficiencies during the acute stage after SAH, and thus only short-term administration of a regular FO dose should be necessary. In fact, a 5-day parenteral perioperative regimen did not increase the occurrence of major postoperative complications in 19 surgically treated SAH patients [13]. Thus, there is good quality evidence to warrant further clinical trials of parenteral pharmaconutrition as an integral component of interventions with omega-3 FAs in SAH patients.

10. Role of inflammation resolution in the growth and rupture of intracranial aneurysms

The role of inflammation in the growth and rupture of intracranial aneurysms (IAs) has been increasingly recognized over the last few decades; however, the specific role of resolution of inflammation in IAs has not yet been considered [76]. Although the pathophysiology of atherosclerosis and the growth and rupture of IAs are distinct, both conditions are mediated by an underlying inflammatory process [77]. The progression of atherosclerotic plaques determines plaque morphology and the risk of rupture. The degree of macrophage infiltration plays a crucial role in the progression of atherosclerotic plaques. Interestingly, IAs have also been recently regarded as a macrophage-mediated inflammatory disease in which prostaglandin E2 and the master transcription factor NF-kB may be crucial drivers of inflammatory signals [78, 79]. It should be remembered that prostaglandin E2 is derived from the long-chain omega-6 FAs ARA and that EPA can inhibit the generation of eicosanoids from ARA and also downregulates the activity of NF-kB [25].

Atherosclerotic plaques readily incorporate omega-3 FAs, and a higher plaque EPA content is associated with a reduced number of foam cells and macrophages, as well as increased plaque stability, as determined by a well-formed fibrous cap [80]. Additionally, signs of defective resolution of inflammation have been identified in atherosclerotic plaques [81]. One major function of SPMs (particularly maresins) is to induce phenotypic conversion of macrophages, which decrease inflammation and promote tissue regeneration [16]. In animal models of atherosclerosis, a traditional Western high-fat diet disrupts the homeostasis of inflammation resolution by nutrigenetic modulation of the 12/15-LOX pathways, thereby inhibiting the generation of protective SPMs [81, 82]. These recent findings in atherosclerosis, particularly the involvement of docosanoids in vascular inflammation, provide biological plausibility that defective resolution of inflammation is implicated in the pathogenesis of IA growth and rupture.

Human beings evolved, and their genetic patterns were established on a diet with an omega-6/omega-3 FAs ratio of 1/1, whereas in current Western diets, this ratio is around 16/1 [83]. Thus, this extreme nutritional imbalance in current Western diets should be seriously considered as a potential aggravating factor for the growth and rupture of IAs [48, 82]. This suggestion may appear somewhat counterintuitive considering the high prevalence of IAs with increased risk of rupture in the Japanese population, which has one of the highest dietary intakes of omega-3 FAs worldwide [48, 84]. However, nutritional deficiency of long-chain omega-3 FAs may not be the only factor associated with defective resolution of inflammation. Inter-individual and ethnic variations in the susceptibility to IA growth and rupture could be related to tissue-specific enzymatic deficiencies in the biosynthetic routes that regulate the resolution of inflammation. However, while defective resolution has already been associated with other chronic

inflammatory diseases, it is not yet known whether enzymatic deficiencies contribute to IA growth and rupture, and this novel hypothesis requires further investigation [85, 86].

11. Final remarks

The vital roles of EPA and DHA in the human body emphasize the evolutionary importance of maintaining efficient functional couplings between chemical and biological systems as well as between the vasculature and the brain [87]. Resolution of inflammation and endogenous neuroprotective signaling are interrelated processes that largely depend on EPA and DHA derivatives. This novel concept may open new avenues for public health interventions and innovative research in IAs and SAH.

Although nutrition has been traditionally viewed as a supportive measure, increasing evidence strongly suggests that a more balanced dietary intake of omega-6 and omega-3 FAs may represent the most efficient means of improving the status of inflammation resolution at the population level [48, 82, 83]. This specific dietary recommendation could contribute to decrease the risk of IAs growth and rupture and the devastating consequences of SAH, along with other important health benefits.

The pathological significance of the loss of brain DHA after SAH has been widely ignored, even though strong preclinical evidence supports the hypothesis that the integrity of the neurovascular unit largely depends on high DHA enrichment. This previously unrecognized pathophysiological process may significantly increase the risk of secondary brain injury following SAH.

The robust demonstration of the clinical efficacy of EPA in patients with chronic CV disease supports the encouraging results obtained in preliminary clinical studies of omega-3 FAs in SAH and warrants a large-scale RCT. It needs to be emphasized that DHA should always be included in neuroprotective interventions, as DHA plays an essential role in neural tissue and is the cornerstone for docosanoid generation. Parenteral pharmaconutrition with FO offers major clinical advantages for the treatment of SAH patients and should also be an integral component of omega-3 FAs interventions during the acute stage of the disease.

The design of future RCTs of omega-3 FAs in SAH should bear in mind a potentially important factor. Clinical approaches that mainly focus on large-artery vasospasm may actually counteract the beneficial effects of omega-3 FAs and other neuroprotective interventions. The main rationale behind this seemingly paradoxical notion is based on both theoretical models and clinical perspectives [3, 31]. An unpublished subgroup analysis of our pilot pharmaconutrition trial of omega-3 FAs showed unexpected differences in the occurrence of cerebral infarction due to DCI between study centers, each of which had different clinical approaches to large-artery vasospasm [13]. In addition, a recently published observational study performed in the UK showed centers that screened for large-artery vasospasm using transcranial Doppler ultrasound (TCD) had poorer inhospital outcomes and similar rates of DCI diagnosis compared to centers that did not [88]. These results support the dissociation between large-artery vasospasm and clinical outcome that has been observed in major phase 3 RCTs in SAH [8]. Therefore, reliance on a surrogate clinical endpoint such as large-artery vasospasm may lead to the adoption of useless or even harmful clinical approaches [88–91]. Indeed, some research centers in Europe do not include TCD ultrasound or endovascular rescue therapy in their treatment protocols for SAH patients [92].

Moreover, it would be clinically meaningful to determine if correlations exist between the omega-3 index and the concentrations of EPA and DHA as well as the status of inflammation resolution in the wall of ruptured and non-ruptured IAs. There may be a real opportunity for a readily implementable and low-cost therapy if the walls of IAs are as responsive to omega-3 FAs as atherosclerotic plaques.

The interplay between omega-3 FAs and widely used drugs (aspirin and statins) that lead to the generation of longer-acting SPM R-epimers provides ample opportunities for future translational approaches in IAs and SAH. Indeed, combined therapies with omega-3 FAs and aspirin or statins could represent a viable, easy-to-implement therapeutic strategy for patients with unruptured IAs.

Parenteral pharmaconutrition with FO could also be a clinically effective intervention for perioperative neuroprotection for patients subjected to other surgeries at high risk of neurological injury, such as carotid endarterectomy, cardiac surgery, or diverse neurosurgical procedures.

In the future, new drug delivery systems capable of carrying synthetic analogues of SPMs could become a viable therapeutic strategy for patients with tissue-specific enzymatic deficiencies in the resolution pathways [93].

Conflict of interest

The authors have no conflicts of interest to declare.

Notes/thanks/other declarations

We express our appreciation to our patients from whom we have learned so much.

Author details

Geisi Saito[1] and Rodrigo Zapata[2*]

1 Department of Anaesthesiology, Asenjo Neurosurgery Institute, Santiago, Chile

2 Neurosurgery Service, Regional Hospital of Rancagua, Hospital Regional Libertador Bernardo O'Higgins, Rancagua, Chile

*Address all correspondence to: rzapata.barra@gmail.com

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