Cardioprotective mechanism of omega-3 polyunsaturated fatty acids

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ABSTRACT

Omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid, are widely regarded as cardioprotective. Several large-scale, randomized clinical trials have shown that dietary intake of omega-3 PUFAs improves the prognosis of patients with symptomatic heart failure or recent myocardial infarction. Therefore, dietary consumption of omega-3 PUFAs is recommended in international guidelines for the general population to prevent the occurrence of cardiovascular diseases (CVDs). However, the precise mechanisms underlying the cardioprotective effects of omega-3 PUFAs are not fully understood. Omega-3 PUFAs can be incorporated into the phospholipid bilayer of cell membranes and can affect membrane fluidity, lipid microdomain formation, and signaling across membranes. Omega-3 PUFAs also modulate the function of membrane ion channels, such as Na and L-type Ca channels, to prevent lethal arrhythmias. Moreover, omega-3 PUFAs also prevent the conversion of arachidonic acid into pro-inflammatory eicosanoids by serving as an alternative substrate for cyclooxygenase or lipooxygenase, resulting in the production of less potent products. In addition, a number of enzymatically oxygenated metabolites derived from omega-3 PUFAs were recently identified as anti-inflammatory mediators. These omega-3 metabolites may contribute to the beneficial effects against CVDs that are attributed to omega-3 PUFAs.

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Introduction

The beneficial effects of n-3 polyunsaturated fatty acids (PUFAs), primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were first recognized in the late 1960s when epidemiological evidence from the Inuit population, who consume an n-3 PUFAs-rich diet, showed that they have a low incidence of myocardial infarction [1]. Subsequently, a large number...
of randomized clinical trials have investigated the efficacy of supplementation with fish oil or omega-3 PUFAs. The GISSI-Prevenzione trial [2] was the first large-scale, randomized trial to provide evidence that dietary supplementation with omega-3 PUFAs had favorable effects on hard clinical end-points in post-myocardial infarction patients. The GISSI-HF trial [3] also showed that omega-3 PUFAs could reduce morbidity and mortality in patients with symptomatic chronic heart failure who were receiving standard treatments, including aspirin, beta-blockers, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, and aldosterone receptor blockers. Further, the JELIS study [4], a randomized large trial conducted in Japan, revealed that treatment with a pharmaceutical preparation of highly purified EPA in addition to statin significantly prevented cardiovascular events in patients with recent myocardial infarction through an apparently cholesterol-independent mechanism. The results of the above-mentioned studies showed that omega-3 PUFAs treatment is safe and well tolerated, and the clinical improvements observed were additive to those of other well-established therapies. Therefore, in a scientific statement from the American Heart Association, the consumption of >1 g/day of omega-3 PUFAs (EPA+DHA; this dose was largely determined from the results of the GISSI-Prevenzione study) was recommended for patients with coronary artery disease to reduce triglyceride (TG) levels, maintain cardiac function, and reduce the risk of coronary heart disease [5]. In addition, many animal studies have demonstrated that omega-3 PUFAs have pleiotropic beneficial effects in the cardiovascular system, including anti-arrhythmic, plasma TG-lowering, anti-thrombotic, anti-atherosclerotic, endothelial relaxation, blood pressure-lowering, anti-inflammatory, and anti-fibrotic effects [6]. In this review, we present recent advances in our understanding of the molecular mechanisms underlying the cardioprotective effects of omega-3 PUFAs.

Modification of the cell membrane milieu by incorporation of omega-3 PUFAs

The cell membrane is composed of phospholipids (PLs) that contain various types of fatty acids. The length and saturation of the fatty acids in these PLs is thought to affect the properties of cell membranes by altering the microdomain “rafts” and “caveolae” that concentrate membrane proteins and lipids and function as signaling platforms. Since omega-3 PUFAs have many double bonds and long-chain carbons, their incorporation into the PLs within a membrane can alter its properties and influence the function of various membrane proteins (Fig. 1), including the suppression of protein kinase C theta signaling and interleukin (IL)-2 production [7], and the disruption of dimerization and recruitment of toll-like receptor 4 [8]. Of note, alteration of the lipid microenvironment in cardiomyocytes through the inclusion of omega-3 PUFAs can modulate ion channel function, leading to anti-arrhythmic effects [6].

Anti-arrhythmic effects of omega-3 PUFAs due to ion channel modulation

The clinical outcomes of several trials, including GISSI-Prevenzione, have suggested that omega-3 PUFAs might prevent the occurrence of sudden cardiac death triggered by lethal arrhythmias. Accumulating evidence from in vivo and in vitro experiments has demonstrated that omega-3 PUFAs exert anti-arrhythmic effects through modulation of myocyte electrophysiology. Omega-3 PUFAs reduce the activity of membrane sodium channels in cardiomyocytes, thus increasing the threshold for membrane potential depolarization [9]. EPA and DHA also modulate the activity of L-type calcium channels, leading to a reduction in free cytosolic calcium ion, which stabilizes myocyte

Fig. 1. The proposed molecular mechanism of cardioprotection by omega-3 PUFAs. Omega-3 PUFAs modulate cell membrane property when incorporated into the phospholipid bilayer and control membrane ion channels to prevent lethal arrhythmia. Also omega-3 PUFAs exert anti-inflammatory and anti-fibrotic effects by modifying NF-κB signaling, the NLRP3 inflammasome, PPARα/γ, GPR120, and TGF-β signaling. NF-κB: nuclear factor-κB; NLRP3: NOD-like receptor family, pyrin domain containing 3; PPARα/γ: peroxisome proliferator-activated receptor α/γ; GPR120: G protein-coupled receptor 120; TGF-β: transforming growth factor-β.
electrical excitability to prevent fatal arrhythmia [10]. EPA blocks the sodium-calcium channel; however, a single amino-acid point mutation in this channel attenuated the inhibitory effect of EPA [11]. These findings suggested that the cardioprotective effect of n-3 PUFAs is mediated by direct interaction with membrane ion channels (Fig. 1).

The anti-arrhythmic effects of omega-3 PUFAs, which occur by blocking various ion channels, are encouraging. In fact, the results of several trials have suggested that dietary supplementation with omega-3 PUFAs might be an effective optional therapy for arrhythmias. However, the FORWARD trial, a prospective, randomized, clinical trial, found that treatment with omega-3 PUFAs over a 6-month period did not reduce recurrent symptomatic atrial fibrillation [12]. In the specific study population with paroxysmal atrial fibrillation (AF), it was concluded that omega-3 PUFAs are not a useful treatment. These results do not exclude the potential anti-arrhythmic effects of prescription omega-3 PUFAs in combination with antiarrhythmic drugs in different populations, such as patients with heart failure; however, validation in prospective trials is required.

The anti-inflammatory effects of omega-3 PUFAs mediated by nuclear receptors, G-protein coupled receptors, and other mechanisms

Acute and chronic inflammation underlie the pathogenesis and progression of various cardiovascular diseases (CVDs), including myocarditis, myocardial infarction, aortic dissection, atherosclerosis, and cardiac remodeling. It is widely believed that the anti-inflammatory properties of omega-3 PUFAs contribute to their cardioprotective effects. In fact, dietary intake of omega-3 PUFAs was reported to decrease the circulating concentrations of inflammatory cytokines such as tumor necrosis factor (TNF), IL-1β, and IL-6, and ameliorate left ventricular functional capacity in non-ischemic dilated cardiomyopathy [13].

EPA and DHA downregulate the expression of inflammation-related genes through inhibition of NF-κB signaling by blocking IκB phosphorylation [14] (Fig. 1) or through the nuclear receptor PPARα/γ [15] (Fig. 1). In addition, omega-3 PUFA is a ligand for GPR120, which attenuates both toll-like receptor 4- and TNF-α-mediated proinflammatory signaling in macrophages [16] (Fig. 1). GPR120, a member of the GPCR family, is also highly expressed in mature adipocytes, and GPR120 stimulation with omega-3 PUFAs was shown to augment GLUT4 glucose transporter expression to promote glucose uptake in adipocytes [16]. Chronic tissue inflammation is a well-known cause of insulin resistance. In a mouse obesity model, the anti-inflammatory and insulin-sensitizing effects of omega-3 PUFAs were shown to be dependent on the expression of GPR120, and administration of a selective agonist improved insulin resistance [17]. NOD-like receptor family, pyrin domain-containing 3 (NLRP3) senses non-microbial danger signals and forms an inflammasome, leading to a sterile inflammatory response in myocardial infarction, ischemia reperfusion injury, and pressure overload-induced cardiac remodeling. Omega-3 PUFAs prevented NLRP3 inflammasome-dependent inflammation and metabolic disorder in HFD-induced diabetic mice [18] (Fig. 1). Moreover, EPA and DHA directly reduced cardiac fibrosis under pressure overload by inhibiting TGF-β-1-induced smad2/3 nuclear translocation, a major pathway involved in the development of cardiac remodeling [19] (Fig. 1). EPA and DHA increased nitric oxide production and promoted subsequent activation of the cyclic GMP/PKG pathway in cardiac fibroblasts. Taken together, these findings suggest that omega-3 PUFAs may have both anti-inflammatory and anti-fibrotic properties.

Novel bioactive lipid mediators of omega-3 PUFAs

Since the 1970s, the beneficial effects of omega-3 PUFAs have been commonly explained to be attributed to either prevention of the conversion of the omega-6 PUFA arachidonic acid (AA) into pro-inflammatory prostaglandins (PGs) and leukotrienes (LTs), or its ability to serve as an alternative substrate, producing less potent mediators, such as 3-series PGs and thromboxanes (TXs) and 5-series LTs (Fig. 2). For instance, the lower incidence of myocardial

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**Fig. 2. Anti-inflammatory effects of omega-3 PUFAs through mediator balance.** The anti-inflammatory effects of omega-3 PUFAs are attributed to (1) prevention of the conversion of AA into PGs and LTs or (2) its ability to function as an alternative substrate to produce less potent mediators. (3) Resolvins, protectins, and maresins, distinct anti-inflammatory and pro-resolving lipid mediators derived from omega-3 PUFAs. AA: arachidonic acid; PGs: prostaglandins; LTs: leukotrienes.
Infarction in a population that consumed a diet rich in omega-3 PUFAs could be due, in part, to a reduction in the formation of the pro-thrombotic prostanoid TXA2 from AA. Moreover, omega-3 PUFAs are metabolized to PGI3, which possess anti-platelet effects, and TXA3, which does not induce platelet aggregation [20].

Liquid chromatography–mass spectrometry (LC–MS)-based lipidomic analyses of murine inflammatory exudates or activated cell supernatants have identified distinct omega-3 PUFA-derived pro-resolving mediators, such as resolvins, protectins, and maresins [21] (Fig. 2). They possess distinct chemical structures and exert their anti-inflammatory effects in a stereospecific manner. These mediators are now generally termed specialized pro-resolving mediators (SPMs). Resolvin E series are synthesized from EPA through the conversion of 18-hydroxyicosapentaenoic acid (18-HEPE) by aspirin-acetylated COX2 or CYP450 monooxygenase. RvE1 actively switches off leukocyte trafficking to the inﬂamed site, promotes the clearance of inflammatory cells and debris, and suppresses cytokine production, thereby leading to resolution of acute inﬂammation [21]. In addition, RvE1 can inhibit platelet aggregation by ADP or TXA2 receptor activation [21].

DHA-derived mediators, such as protectins, resolvin D-series, and maresins, are generated by 15-LOX in humans or by 12/15-LOX in mice. PD1 exhibits protective effects against brain ischemia, resistance to retinal oxidative injury, and protection against renal ischemia/reperfusion injury [21]. RvD1 is shown to be protective in various disease models, such as insulin resistance, atherosclerosis, ischemia reperfusion, and others [21]. In addition, RvD2 could reduce the damage and subsequent scarring of kidneys after ischemia/reperfusion [21]. PD1 and RvD1 have been shown to reduce pathogenic neovascularization in murine oxygen-induced retinopathy [21]. Furthermore, a recent report showed that administration of PD1 isomer (PDX) improved oxygen-induced retinopathy [21]. Furthermore, a recent report showed that treatment with RvD1 accelerated the resolution of acute inflammation following myocardial infarction by promoting the production of SPM in the spleen and switching to anti-inflammatory M2 macrophages in the left ventricle, which prevented cardiac fibrosis and preserved cardiac function.

PUFAs are oxidized by CYP450 monooxygenase to epoxides, which function as potent lipid mediators in the cardiovascular system. The epoxycosatrienoic acids (EETs) generated from AA induce vasodilation, stimulate angiogenesis, and protect the heart from ischemia/reperfusion injury. CYP450 monooxygenase also converts EPA and DHA to epoxycosatetraenoic acids (EpETEs) and epoxycosapentaenoic acids (EpDPAs), respectively, which function as lipid mediators similar to EET. By activating BK channels, 17(18)-EpETE dilates human pulmonary arteries and 16(17)-EpDPA activates rat coronary smooth muscle [21]. Omega-3 PUFA-derived epoxides possess anti-inflammatory properties in the setting of CVD. The increased EpETE and EpDPA levels following dietary administration of omega-3 PUFAs reduced the renal markers of inflammation in angiotensin II-dependent hypertension [21]. EpETEs and EpDPAs protect the heart through their anti-arrhythmic actions. Recent studies are focusing on the development of stable epoxide bioisosteric analogs and soluble epoxide hydrolase inhibitors that can stabilize epoxides by inhibiting their hydrolysis to the corresponding diols in order to potentiate their functional effects and for use as novel therapeutics against CVD [22].

There is accumulating evidence demonstrating that SPMs directly exert cardioprotective actions in vivo. Keyes et al. [25] reported that administration of RvE1 attenuated the infarct size in rats subjected to ischemia/reperfusion injury. The results of this study suggested that RvE1 directly affected cardiomyocytes and protected against cardiac injury. In addition, Kain et al. [26] showed that treatment with RvD1 accelerated the resolution of acute inflammation following myocardial infarction by promoting the production of SPM in the spleen and switching to anti-inflammatory M2 macrophages in the left ventricle, which prevented cardiac fibrosis and preserved cardiac function.

Atherosclerosis is now commonly recognized as an unresolved inflammatory disease involving the vascular wall. Therefore, it is assumed that the pathogenesis of atherosclerosis involves a decrease in SPM production; thus, SPM replacement could control the local inflammatory response and improve the atherosclerotic changes. One study showed that 12/15-LOX is protective against atherosclerosis through the production of SPMs including RvD1 and PD1, which could attenuate the local inflammatory response in macrophages and endothelial cells [23]. Hasturk et al. found that oral-topical application of RvE1 reduced atherogenesis induced by both diet and periodontal inflammation and could prevent the systemic inflammatory response and aortic atherosclerosis in the absence of periodontitis [24].

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Fig. 3. An EPA metabolite, 18-HEPE, from cardiac macrophages rich in omega-3 PUFAs prevents cardiac remodeling under pressure overload. Activated cardiac fibroblasts, namely myofibroblasts, produce pro-inflammatory mediators that facilitate cardiac macrophage activation. Cardiac fibroblasts can be activated directly by pressure overload or secondarily by inflammatory mediators released from activated inflammatory cells. EPA-enriched macrophages generate an 18-HEPE-rich milieu in the heart, thereby discontinue the profibrotic feed-forward loop that is involved in the development of cardiac fibrosis under pressure overload.

EPA: eicosapentaenoic acid; 18-HEPE: 18-hydroxy eicosapentaenoic acid.
Novel beneficial effects of a macrophage-derived EPA metabolite, 18-HEPE, on cardiac remodeling

Recently, we identified a novel anti-inflammatory and anti-fibrotic EPA metabolite, 18-HEPE, and elucidated the mechanism underlying omega-3 PUFA-mediated prevention of cardiac remodeling under pressure overload [29] (Fig. 3). Kang et al. developed a transgenic mouse expressing the Caenorhabditis elegans fat-1 gene, which encodes an omega-3 desaturase that converts omega-6 PUFAs to omega-3 PUFAs [30]. Fat-1 mice showed enrichment of omega-3 PUFAs in almost all cells and tissues, and displayed resistance to numerous inflammatory diseases, including colitis, pancreatitis, osteoarthritis, atherosclerosis, obesity-linked insulin resistance, and some cancers [31]. Compared to wild-type mice, fat-1 mice subjected to pressure overload showed sustained heart function and reduced cardiac remodeling (fibrosis) without any difference in cardiac hypertrophy. Examination of bone marrow transplantation revealed that enrichment of omega-3 PUFAs in bone marrow-derived cells (mostly macrophages), but not in cardiac cells, was responsible for the anti-fibrotic phenotype observed in fat-1 mice subjected to pressure overload. LC-MS/MS-based lipidomics revealed that high levels of 18-HEPE, an EPA metabolite, were produced by fat-1 transgenic macrophages, and 18-HEPE suppressed IL-6 production from cardiac fibroblasts in nanomolar range (Fig. 3). Of note, dietary intake of EPA ethyl ester (2700 mg/day) significantly increased the plasma concentration of 18-HEPE. Furthermore, administration of 18-HEPE prevented cardiac dysfunction, macrophage infiltration, and cardiac fibrosis after transverse aortic constriction (TAC), indicating the therapeutic potential of 18-HEPE for cardiac remodeling under pressure overload [29].

Conclusions

Not all omega-3 PUFAs trials have shown reductions in CVD; however, several adequately powered observation and intervention trials have strongly supported the efficacy of omega-3 PUFAs for the prevention of CVD. Furthermore, experimental studies have revealed multiple underlying molecular mechanisms, including membrane modification, attenuation of ion channels, regulation of pro-inflammatory gene expression, and production of lipid mediators. It remains unclear which mechanism contributes the most to the cardioprotective effects of omega-3 PUFAs observed in vivo; however, the pleiotropic anti-inflammatory effects of omega-3 PUFAs could be valuable, especially in the setting of atherosclerosis and cardiac remodeling. Although further work is needed to clarify the molecular relationship between omega-3 PUFAs and cardiac physiology/pathology, it might be useful to consider bioactive omega-3 PUFA-derived metabolites, such as 18-HEPE, as endogenous anti-inflammatory molecules and potential new therapeutic targets for CVD.

References


Conflict of interest

The authors have no conflict of interest to disclose.


