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Review

Non-enzymatic cyclic oxygenated metabolites of omega-3 polyunsaturated fatty acid: Bioactive drugs?

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ABSTRACT

Non-enzymatic oxygenated metabolites derived from polyunsaturated fatty acids (PUFA) are formed *in vivo* through free radical reaction under oxidative stress conditions. It has been over twenty-five years since the discovery of cyclic oxygenated metabolites derived from arachidonic acid (20:4 n-6), the isoprostanes, and since then they have become biomarkers of choice for assessing *in vivo* OS in humans and animals. Chemical synthesis of n-3 PUFA isoprostanoids such as F_3-Isoprostanes from eicosapentaenoic acid (20:5 n-3), and F_4-Neuroprostanes from docosahexaenoic acid (22:6 n-6) unravelled novel and unexpected biological properties of such omega-3 non-enzymatic cyclic metabolites as highlighted in this review.

1. Introduction

Docosahexaenoic acid (22:6 n-3, DHA) and eicosapentaenoic acid (20:5 n-3, EPA) are the major n-3 polyunsaturated fatty acids (PUFAs) of marine fish oil. Evidences from epidemiological studies, clinical trials, animal and cellular experiments showed fish oil and specifically n-3 PUFA, to have beneficial effects in numerous diseases [1]. Paradoxically, due to the abundance of double bonds in the structure of EPA and DHA, they are prone to free radical attack and can undergo spontaneous non-enzymatic peroxidation to generate cyclic oxygenated metabolites [2] together with acyclic metabolites. The excessive release of these EPA and DHA metabolites [3] are related to neurological disorders such as Alzheimer's disease, Parkinson's disease and mild cognitive dysfunction since their elevations in plasma were reported to correlate with disease progression [4,5].

Under oxidative stress (OS) condition, PUFA precedes to peroxidation and numerous cyclic oxygenated products are released [4,6,7], the most famous being the isoprostanes and the isofurans. For example, arachidonic acid (20:4 n-6, AA) under OS generates 64 isomers of F_2-isoprostanes (F_2-IsoPs) [8] (Scheme 1) and 258 isomers of isofurans (IsoFs) [9], EPA generates 96 isomers of F_3-isoprostanes (F_3-IsoPs) and DHA generates 128 isomers of F_4-neuroprostanes (F_4-NeuroPs) [10] and 512 isomers of neurofurans (NeuroFs) [11].

In the nineties, measurement of 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) were one of the few biomarkers to assess OS in biological systems. However, common contentious suggested F_2-IsoPs as more robust biomarker [12] in assessing endogenous OS in humans [7], animals models [12] and in biological fluids [13,14]. These molecules are oxidized *in situ* on the phospholipid membranes and hydrolyzed via phospholipase A_2 (PLA_2) and platelet activating factor acetylhydrolase into the free form, and released in tissues and systemic circulation. Among these metabolites, some have been commonly, and in some cases routinely measured as OS biomarkers related to vascular systems and neurodegeneration [6,11].

The discovery and study of notable isoprostanoids (Scheme 1) have provided a major step forward in the field of free radical research. The quantification of these molecules has opened up new areas of investigation regarding the role of free radicals in human

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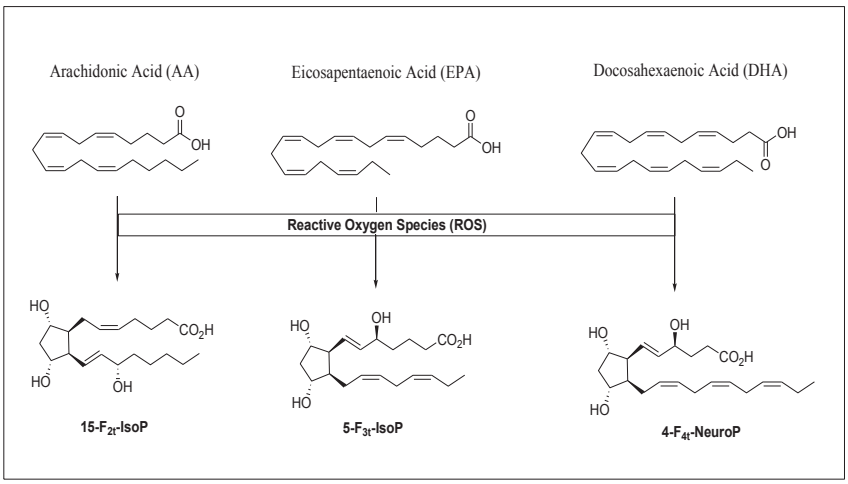

Scheme 1. Structures of bioactive isoprostanoids F_2-IsoPs, F_3-IsoPs and F_4-NeuroPs released from their respective PUFA precursors.

physiology and pathology, and appears to be the most useful tool currently available to explore the role of endogenous lipid peroxidation in human diseases. However, as explained below such molecules are not simple biomarkers but also exert bioactive properties.

Evidences in favour of the bioactive role of isoprostanoids from n-6 PUFA were shown in various biological systems [14–16]. In particular, 15-F_{2t}-IsoP and 15-E_{2t}-IsoP are known to possess potent biological activities ranging from the effects on vascular and bronchial smooth muscle, endothelium function, platelet function and to cell proliferation [4] (Scheme 1).

This review will focus solely on the non-enzymatic cyclic oxygenated metabolites of n-3 PUFA and their potential biological actions in human health and diseases.

2. Biological activities of n-3 PUFA metabolites

The understanding of the role of n-3 PUFA peroxidation in the pathogenesis of various diseases continues to expand but the biological activity of their cyclic oxygenated metabolites remains unclear. The bioactive effects of non-enzymatic products from n-3 PUFA have been largely undermined by investigators and remain unexplored. The reasons for this paucity of investigation could be due to the false idea that the rate of non-enzymatic PUFA oxidation *in vivo* is negligible, and/or to the previously held idea that any form of lipid peroxidation is undesirable as it is unconditionally toxic. Moreover, not all of these metabolites are commercially available and needs to be custom synthesized.

The biological roles of oxygenated metabolites from the peroxidation of n-3 PUFA mainly emphasise on enzymatic pathways, especially on their anti-inflammatory activities and the reduction of pro-inflammatory eicosanoids stemming from AA [7,18]. For example, lipoxygenase regulate metabolites such as resolvins, protectins and maresins [19,20] and such compounds have shown a large range of potent anti-inflammatory activities. Nevertheless, recent studies showed that isoprostanoids *per se* derived from n-3 PUFA (mainly from EPA, DHA and α-linolenic acid (C18:3 n-3, ALA) are new actors to be considered [5,17,20,21] suggesting that bioactive role of oxygenated n-3 PUFA is not limited to those released through enzymatic pathway.

Sethi's group demonstrated for the first time that DHA in OS environment regulates anti-inflammatory activities [22]. In this study, the authors demonstrated that pre-incubation of endothelial cells with oxidized EPA and DHA (generated by the reaction of copper sulphate) reduced adhesion of monocyte cells to endothelial cells while the native EPA and DHA had no effect. The authors hypothesized that the reduced expression of adhesion molecules such as VCAM-1 by the endothelial cells decreased the interaction of phagocyte/endothelial cells through the action of anti-inflammatory property of the unknown forms of oxidized EPA and DHA metabolites. Follow up to this, the same group evidently showed the reduction of pro-inflammatory cytokines MCP-1 (a monocyte chemoattractant protein) in the endothelial cells when exposed to oxidized n-3 PUFA [23]. Following these seminal works, others studies clearly demonstrated biological properties of n-3 PUFA were dependent on their peroxidation [24–29] but the exact nature of the bioactive molecules was not elucidated.

In order to identify the oxidized compounds for the biological role of n-3 PUFA, it is necessary to design studies that use a single molecule of interest. Nonetheless, only a few research groups have successfully synthesized cyclic oxygenated metabolites from n-3 PUFA [30–32] such as F_3-IsoPs from EPA [33] and F_4-NeuroPs from DHA [34], and their availability allowed a better understanding of their biological roles.

2.1. Metabolites of EPA

Over a decade ago, one study highlighted that unlike the 15-F_{2t}-IsoP derived from AA, 15-F_{3t}-IsoP from EPA does not activate the platelet aggregation [35]. This notable difference of activity between almost similar cyclic oxygenated products derived from n-6 PUFA and n-3 PUFA suggests a very subtle structure-activity relationship [7]. More recently, Jamil et al. [39] investigated the ability of another isomer of F_3-IsoPs, the 5-F_{3t}-IsoP (Scheme 1) which shown to regulate glutamatergic neurotransmission. Hence, 5-F_{3t}-IsoPs could have important pharmacological implications in neurology since EPA is rich in the brain and retina. Glutamate serves as the primary excitatory neurotransmitter in several vertebrate retinal cells, including ganglion cells. The group also investigated the modulatory role of 5-*epi*-5-F_{3t}-IsoP on K^+-induced glutamate release in isolated bovine retina. They found that 5-*epi*-5-F_{3t}-IsoP attenuates K^+-induced [^{3}H] D-aspartate release in a concentration-dependent manner and indicated that the mechanism involved is due to, in part pre-junctional prostanoid EP1-receptors activation. This result displays the beneficial role of 5-*epi*-5-F_{3t}-IsoP by reducing excitatory neurotransmitter release, thereby retarding the progression of ocular neuropathic disease.

A_3/J_3-IsoPs, the EPA-derived cyclopentenone isoprostanoids were also identified for their biological qualities *in vivo* under OS

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