Non enzymatic metabolites of polyunsaturated fatty acids: friend or foe

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Abstract — Under condition of oxidative stress, free radical mediated peroxidation of polyunsaturated fatty acids generates in vivo, cyclic metabolites like the isoprostanes, neuroprostanes, dihomo-isoprostanes, isofuranes among a large number of key products which participate in many pathophysiological processes. These metabolites display a wide range of biological actions, and some of them are now the most reliable indicators of oxidative stress in humans. In this review, we will discuss several key points of our understanding of those cyclic polyunsaturated fatty acids derivatives, going from multi-step syntheses, analytical chemistry and biological activities.

Keywords: biomarkers / polyunsaturated fatty acid / radical peroxidation / isoprostanes / neuroprostanes / isofurans / total synthesis


Mots clés : biomarqueurs / activités biologiques / peroxydation lipidique / acides gras polyinsaturés / isoprostanes / neuroprostanes / isofuranes

1 Introduction

In mitochondrial and microsomal membranes, molecular dioxygen can be transformed into several reactive oxygen species (ROS). Some of them can be seen as low reactive like the superoxide anion (O$_2^-$) which confers them their quality of regulators of several important biological functions. Under condition of oxidative stress (OS), the radical hydroxide (HO$^-$), known to be preferentially formed through the Fenton and Haber-Weiss reactions (Fig. 1), is a more reactive species (along with peroxyle HOO$^-$) and has been implicated in the radical-mediated peroxidation of polyunsaturated fatty acids (PUFA) (Magder, 2006).

A living mammalian organism has many endogenous substances or enzymes able to maintain the ROS in a steady concentration or to trap the free radicals. In addition, the diet can supply exogenous anti-oxidant substances (like vitamins for example). However during particular circumstances if the overwhelming production of ROS cannot be countered by antioxidant system, important biological molecules (lipids, proteins, nucleic acids…) will undergo radical mediated modifications.

In this review we will focus on lipids and especially on radical induced peroxidation of PUFAs by ROS (Jahn et al., 2008). The hydroxide radical is able to abstract a hydrogen atom from a methylene group surrounded by the two-double bonds in PUFAs and thus to initiate the lipid peroxidation process. The progression of lipid peroxidation will promote several changes, loss of physiological function of cell membrane, inactivation of enzymes and further biochemical changes will have both desirable and undesirable effects. For example they are in part responsible of disorders like stroke, myocardial infarction, inflammatory disorder, cancers, diabetes, neurodegenerative diseases (Colvaiti and Finkel, 2005; Il’yasova et al., 2008; Janssen et al., 2008; Steinberg et al., 1989).

The most abundant PUFA in human organism is arachidonic acid (AA, C20:4 n-6) (Morrow et al., 1990; Morrow and Roberts, 1994). Linked to phospholipids in the membranes, it is released in the free form by phospholipase A2 (PLA$_2$) and

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then can be used by cyclooxygenase enzymes to generate the biologically relevant prostaglandins (PGs) or the leukotrienes via lipoxygenase activity.

In 1990, Morrow et al. highlighted *in vivo* formation in human of the isoprostanes (IsoPs), isomeric compounds of the PGs, by a non enzymatic mechanism involving the free radical mediated peroxidation of AA. The IsoPs synthesized in the membranes of the cell by peroxidation of the still esterified AA. A large number of diastereoisomers can be generated and then can be released by a specific PLA2 (Stafforini et al., 2006).

The lipid peroxidation of AA begins with the abstraction of a hydrogen atom by HO·, in one of the 3 bis-allylic positions at C-7, C-10 or C-13. The pentadienyle radical formed reacts with molecular dioxygen to form a peroxy radical which undergoes two consecutive 5-exo-trig cyclizations and the subsequent formed carbon centered allylic radical reacts one more time with dioxygen to generate a hydroperoxide intermediate after abstraction of another H-atom (Fig. 2). Finally, partial and/or complete reductions yield the whole family of IsoPs with several type (A, D, E, F, J) and series (5, 8, 12, 15). Isofurans (IsoFs) metabolites containing furan ring structures can also be formed in preference but not only, from AA, when oxygen tension is elevated (Fessel et al., 2002). This was evidently shown in pigs, in which exposure of high oxygen tension augmented IsoFs production and reduced IsoPs production in the brain (Solberg et al., 2012).

The increase of the IsoP concentration was demonstrated in several pathologies as for example in neurodegenerative and cardiovascular diseases, and IsoPs are now considered as the standard markers of the lipid peroxidation. Their quantification in urine and plasma allows a precise, non-invasive and representative measure of OS (Mas et al., 2008; Michel et al., 2008; Milne et al., 2007; Vigor et al., 2014).

The quantification processes were developed due to the availability of synthetic compounds provided by organic chemists who are able to produce large amount of pure compounds (Jahn et al., 2008). Furthermore, molecules becoming
commerciantly available were studied and since then their biological roles were also uncovered (Galano et al., 2015). For example, Morrow et al. (1990) showed that the 15-F_{2}-isoP injected in a rat kidney, in the peripheral vein or directly in the kidney, allowed the reduction of the blood pressure and the rate of filtration. Other isoPs thus have biological activities which confer them a role of mediator in a context of OS (Brown and Marnett, 2011).

In mammals, this prolific pathway of peroxidation can occur with different PUFAs (Fig. 3), like docosahexaenoic acid (DHA, C22:6 n-3) enriched in the grey matter and retina, which yields the neuroprostanoids (NeuroPs) (Nourooz-Zadeh et al., 1998; Roberts, 1998) and neurofurans (NeuroFs) (Fessel et al., 2010). Adrenic acid (AdA, C22:4 n-6) present in plants (De La Torre et al., 2015, 2014; Song et al., 2008; VanRollins et al., 2008). In plants, the phytoprostanoids (PhytoPs) were described resulting from the radical peroxidation of α-linolenic acid (ALA, C18:3 n-3) (Imbusch and Mueller, 2000).

The lovely story of these molecules will be reported in this review. Herein you can find several outcomes in terms of syntheses, diagnosis and biological activities, gathering twenty five years of our group research, from our organic chemistry knowledge through smart multi-step synthesis strategies of isoP derivatives, to our fruitful collaborations with biologists and clinicians around the world (Galano et al., 2013, 2015).

2 Chemical synthesis

We have reported recently a simple and highly stereocонтrolled strategy toward the total synthesis ofIsoP derivatives based on a bicyclic α, β-epoxy ketone intermediate (Fig. 4) (Oger et al., 2008).

The use of a bicyclo[3.3.0]octene scaffold permitted stereodirectiof reagents allowing the facile 1,3-cis diol unit introduction via stereoselective epoxidation, diastereoselective ketone reduction, and regioselective epoxide opening otherwise not attainable with a simple cyclopentene framework.

Fig. 3. Metabolites resulting of the radical peroxidation of PUFAs.

Fig. 4. Two key bicyclic alcohols.

The lateral chains were plugged by HWE and Wittig reactions, the allylic alcohol reduced and the final deprotections allowed us to validate this flexible strategy and to access IsoPs; dihomo-IsoPs; NeuroPs (Figs. 5 and 6) (Brinkmann et al., 2010; Oger et al., 2010, 2012).

3 New biomarkers

3.1 Phytoprostanoids

Supplementation with eicosapentaenoic acid (EPA; C20:5 n-3) and DHA has been reported to reduce lipid peroxidation products (F2-IsoPs) formed from AA in healthy humans, as well as in patients with conditions associated with OS. While the shorter chain PUFA, ALA, is ubiquitous in plant cells and can serve as a precursor to EPA and DHA to human; its conversion to C20 and C22 PUFA seems to be inefficient. As described above, ALA can also undergo free radical oxidation, forming PhytoPs in all plants and leading to the accumulation of high concentrations of PhytoPs in plant pollens (Mariani et al., 2007; Traidl-Hoffmann et al., 2005). In a recent study with healthy male volunteers, Barden et al. (Barden et al., 2009) examined the effect of ALA supplementation on F1-PhytoPs and F2-IsoPs concentrations in plasma and urine.

The study protocol was as follows: thirty-six non smokers, 20–65 years of age, consumed 9 g/day of either flaxseed oil (62% ALA, 5.4 g/day) or olive oil (placebo) for 4 weeks in a parallel design. At baseline and after 4 weeks of supplementation, blood and a 24-h urine sample were collected for determination of concentrations of F1-PhytoPs and F2-IsoPs, and selected plasma fatty acids.

Compared with the group supplemented with olive oil, the flaxseed oil group showed significantly higher levels of ALA in plasma phospholipids (p < 0.0001), as well as significant elevations of F1-PhytoPs in plasma (p = 0.049) and urine (p = 0.06). Flaxseed oil did not affect plasma or urinary F2-IsoPs levels. The higher plasma F1-PhytoP concentration in the flaxseed oil group most likely resulted from the increased plasma concentration of the ALA substrate and/or the greater F1-PhytoP content of the flaxseed oil (Fig. 7).

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The use of a bicyclo[3.3.0]octene sca

\[ \text{docosahexaenoic acid (DHA)} \]

\[ \text{adrenic acid (AdA)} \]

\[ \text{α-linolenic acid (ALA)} \]

\[ \text{Phytoprostanes} \]

\[ \text{Dihomo-Isoprostanes} \]

\[ \text{Neuroprostanes} \]

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plants. For example, Collado-Gonzales et al. have proposed a quick and accurate new analytical method by UHPLC–QqQ-MS/MS which is able to identify free PhytoPs, in olive and refined sunflower oils (Collado-Gonzalez et al., 2015). The recovery provided high extraction efficiencies ranging from 102.90% to 140.64% using Strata-XAW cartridge. The intra-day and inter-day variations for all target compounds ranged from 2.24% to 13.64% and 0.01% to 13.69%, respectively, and the accuracies for these parameters varied from 80.33% to 119.64% and from 80.34% to 119.90%, respectively. Results obtained reflect that refined sunflower presented more series of PhytoPs and a 20 and 8-fold higher quantity of PhytoPs than two types of olive oil: extra virgin olive oil and olive oil. The manufacture process could be the key for the different PhytoP production since most of the plant oils are subjected to a refining treatment. The results of this analysis performed in a single assay per sample are obtained within eight minutes, in addition, these advantages are linked to lower expenses in solvents.

From agronomical point of view in connection with food science and technology, future research is required to study how the production of PhytoPs is affected by different types of abiotic stress on olive oil and other plant oils. Nutritionally speaking, additional studies would be necessary to know the physiological effects of the PhytoPs in humans, since they show very similar structures than IsoPs and PGs, relevant bioactive compounds at physiological level.

Carrasco et al. studied almonds which have favourable contents of PUFA especially ALA (Carrasco-Del Amor et al., 2015). This study represents the first approach to the quantitative and qualitative determination of the PhytoP profile in 11 almond cultivars under different agronomic conditions (conventional versus ecological, rain-fed versus irrigated). In the kernels have been identified 9-F_{1t}-PhytoP, 9-epi-9-F_{1t}-PhytoP, ent-16-epi-16-F_{1t}-PhytoP, 9,epi-9-D_{1t}-PhytoP, 16-B_{1}-PhytoP and 9-L_{1}-PhytoP. The total PhytoP content was in the range of 4.0 to 23.8 ng per 100 g. F_{1t}-PhytoPs predominated in all almond cultivars. L_{1}-PhytoPs
were minor components while D1-PhytoPs were only detected in cultivars ‘Colorada’ and ‘Avellanera’. The PhytoP profile varied greatly depending on the genotype, but was also affected by factors such as the agricultural system (conventional or ecological) and irrigation. The ecological system promoted the synthesis of D1-PhytoPs. Almonds from rain-fed trees had lower individual and total PhytoP concentrations than those under irrigation, even though non-irrigation led to the detection of the 16-F1-PhytoP. Consequently, irrigation and ecological techniques applied to almonds could be considered as actions to enhance their PhytoP content and hence their potential beneficial effects on human health.

PhytoPs have been studied in several plant species, but information regarding the natural occurrence of this large family of biologically active oxidized lipids in macroalgae is still scarce. Barbosa et al. (2015) studied the free PhytoP composition of 24 macroalgae species belonging to Chlorophyta, Phaeophyta, and Rhodophyta using an UHPLC-QqQ-MS/MS method (Barbosa et al., 2015). The PhytoP profiles varied greatly among all samples, F11-PhytoP and L1-PhytoP being the predominant and minor classes, respectively. No correlation between the amounts of ALA in alga material and PhytoP content was found. Therefore, it was hypothesized that the observed variability could be species-specific or result from interspecific interactions. This study provides new insight about the occurrence of PhytoPs in macroalgae and opens doors for future exploitation of these marine photosynthetic organisms as sources of potentially bioactive oxylipins, encouraging their incorporation in food products, nutraceutical and pharmaceutical preparations for human health.

3.2 Neuroprostanes and isofurans

OS may contribute to the pathogenesis of pre-eclampsia, a life-threatening disorder of pregnancy that adversely affects the mother and the baby (Barden et al., 2004). In 1996 Barden et al. showed that plasma F2-IsoP level raises in proteinuric pre-eclampsia (Barden et al., 1996). In a recent study IsoFs, F3-NeuroPs and F2-IsoPs were quantified in maternal plasma and cord blood of women with pre-eclampsia and normal pregnancies (Barden et al., 2012). Women with pre-eclampsia had significantly elevated maternal IsoFs and F2-NeuroPs, but no F2-IsoPs. Cord blood F2-NeuroPs were elevated among neonates of women with pre-eclampsia. Interestingly, cord blood IsoFs were approximately 5-fold higher than those found in maternal plasma. This could reflect the oxidative challenge presented at birth, when there is transition from a relatively low intra-uterine oxygen environment to a significantly higher extra uterine oxygen environment. Maternal F2-NeuroPs were not significantly correlated with cord blood F2-NeuroPs in pre-eclamptic and in normal pregnancies, suggesting the origin of cord F2-NeuroPs may be independent of maternal plasma. In normal pregnancy birth weight was negatively related to maternal F2-IsoPs, IsoFs and F4-NeuroPs.

The brain is vulnerable to oxidative insult because of high oxygen requirements for its metabolism and high PUFA composition, in particular DHA. Thus F2-IsoPs are considered as specific markers of brain OS. Aneurysmal subarachnoid hemorrhage (aSAH) and traumatic brain injury (TBI) are associated with devastating central nervous system (CNS) injury. In two case-controlled studies we have shown a significant increase in cerebrospinal fluid (CSF) IsoFs in aSAH and TBI patients compared with their respective age- and gender-matched controls. aSAH patients also had significantly increased levels of CSF F2-NeuroPs and F2-IsoPs. Patients with TBI had significantly increased CSF F2-NeuroPs but F2-IsoPs were similar to control (Corcoran et al., 2011). These data confirm that CNS injury, in case of aSAH or TBI, results in increased OS and as DHA is the brain major PUFA, F3-NeuroPs levels in CSF could be a much more specific indicator of neurological dysfunction than F2-IsoP. Hsieh et al. have shown that increased F2-NeuroPs in CSF of patients with aSAH correlated with poor neurological outcome (Hsieh et al., 2009). They suggested that F2-NeuroPs might be more useful than F2-IsoPs in CSF to predict outcome and interpret the role of hemorrhage in aSAH. Although Farias et al. showed increased F2-IsoPs during rat brain ischemia, the F2/D2-IsoPs were increased to a greater extent, suggesting the latter may better markers of OS in brain ischemia (Farias et al., 2008).
The anti-atherogenic effects of n-3 (omega) fatty acids, EPA and DHA are well recognized but the impact of dietary intake on bioactive lipid mediator profiles remains unclear. Such a profiling effort may offer novel targets for future studies into the mechanism of action of omega 3 fatty acids. Gladine et al. studied the impact of DHA supplementation on the profiles of PUFA oxygenated metabolites and their contribution to atherosclerosis prevention (Gladine et al., 2014). A special emphasis was given to the non-enzymatic metabolites knowing the high susceptibility of DHA to free radical-mediated peroxidation and the increased OS associated with plaque formation. Atherosclerosis prone mice (LDLR<sup>−/−</sup>) received increasing doses of DHA (0, 0.1, 1 or 2% of energy) during 20 weeks leading to a dose-dependent reduction of atherosclerosis ($R^2 = 0.97$, $p = 0.02$), triglyceridemia ($R^2 = 0.97$, $p = 0.01$) and cholesterolemia ($R^2 = 0.96$, $p < 0.01$). Targeted lipidomic analyses revealed that both the profiles of EPA and DHA and their corresponding oxygenated metabolites were substantially modulated in plasma and liver. Notably, the hepatic level of F<sub>2</sub>-NeuroPs was strongly correlated with the hepatic DHA level. Moreover, unbiased statistical analysis including correlation analyses, hierarchical cluster and projection to latent structure discriminate analysis, revealed that the hepatic level of F<sub>2</sub>-NeuroPs was the variable most negatively correlated with the plaque extent ($p < 0.001$) and along with plasma EPA-derived diols, was an important mathematical positive predictor of atherosclerosis prevention. Thus, oxygenated n-3 PUFAs, and F4-NeuroPs in particular, are potential biomarkers of DHA-associated atherosclerosis prevention. While these may contribute to the anti-atherogenic effects of DHA, further in vitro investigations are needed to confirm such a contention and to decipher the molecular mechanisms of action.

3.3 Dihomo-isoprostanes

Rett syndrome (RTT) is a pervasive abnormality of development affecting almost exclusively females, which is included among the autism spectrum disorders. RTT is caused in up to 95% of cases by mutations in the X-linked methyl-CpG binding protein 2 (MeCP2) genes (De Felice et al., 2012). Although over 200 different MeCP2 mutations have been reported to cause RTT, nine most frequent ones (hotspot mutations) are known to comprise more than three quarters of all the reported pathogenic mutations. The disease shows a wide phenotypical heterogeneity, with at least 4 distinct major clinical presentations, i.e., typical, preserved speech, early seizure variant, and congenital variant. Clinical evidence indicates that F<sub>2</sub>-IsoPs and F<sub>4</sub>-NeuroPs are involved in the intimate pathogenetic mechanisms of RTT. Plasma levels of free F<sub>2</sub>-IsoPs are significantly higher in the early stages of RTT, as compared with the late natural progression of typical RTT. Until recently it was thought that the predominant central nervous system damage in RTT occurred in gray matter. However, the relative abundance in myelin of the precursor AdA and the increased level of F<sub>2</sub>-dihomo-IsoPs, strongly confirm an early and severe damage to the brain white matter as suggested by previous brain MRI evidence. Thus F<sub>2</sub>-dihomo-IsoPs can be considered early markers of lipid peroxidation in RTT (De Felice et al., 2011). F<sub>4</sub>-NeuroPs also appear to be important biomarkers in RTT. Plasma F<sub>4</sub>-NeuroP levels correlate with disease severity in RTT and are significantly related to neurological symptoms severity, mutation type and clinical presentation. Therefore, F<sub>4</sub>-NeuroPs may play a major role along the biochemical pathway from MeCP2 gene mutation to clinical evidence, proving that a DHA oxidation process occurs.

3.4 Dihomo-isofurans/neurofurans

Neurofurans (NeuroFs) and dihomo-isofurans (dihomo-IsoFs) are produced in vivo by non-enzymatic free radical pathways from DHA and AdA, respectively. As these metabolites are produced in minute amounts, their analyses in biological samples remain challenging. We performed syntheses of NeuroFs and dihomo-IsoFs (Fig. 8), thanks to an enantiomerically enriched intermediate, which allowed, for the first time, access to both families: the alkenyl and enediol (De La Torre et al., 2015, 2014).

Owing to this formation, quantitation of specific NeuroF and dihomo-IsoF in biological samples was attainable and we reported the presence of 4(RS)-ST-$\Delta^5$-8-NeuroF and 7(RS)-ST-$\Delta^8$-11-dihomo-IsoF in rat brain and heart tissues. It is also the first report to show concentration of known NeuroP and dihomo-IsoP in the heart tissue. The concentration difference of 4(RS)-ST-$\Delta^5$-8-NeuroF between the heart and the brain indicates that it is a robust indicator for macro- and micro-vascular function, considering disparate in situ oxygen tension of the tissues. These DHA and AdA metabolites are presently in testing for various pathological models as OS biomarkers and bioactive compounds.

4 New bioactive lipids

Isoprostanes and analogs are not only biomarkers of lipid peroxidation but also mediators of oxidative injury.

4.1 Isoprostanes

IsoPs are vasoconstrictors in many species and various vascular beds, modulate platelet activity and monocyte adhesion, and induce proliferation of endothelial and smooth muscle cells. IsoPs mediate their biological effects by activation and/or inhibition of several prostanoid receptors, among them the thromboxane receptor (TP), prostaglandin F<sub>2</sub> receptor (FP),
prostaglandin E2 subtype 3 receptor (EP3), prostaglandin D2 subtype 2 receptor (DP2) and by activation of the peroxisome proliferators activated receptor gamma (PPAR γ) (Milne et al., 2011).

The vasomotor action of 15-F2t-IsoP has been investigated in isolated human saphenous and umbilical veins, in bronchial, radial and internal mammary arteries, and in pulmonary vasculature as well as placental and maternal vessels. In contrast to 15-F2t-Isop, 5-F2-IsoP-series do not contribute to the vasocstriction mediated by IsoP. Besides vasoconstriction and platelet activation, IsoPs also enhance the vascular reperfusion damage after myocardial infarction; pioneering cardiac smooth muscle apoptosis and scar formation. In this scenario, formation of collateral and new vasculature outgrowth is essential for cardiac function recovery. The complex interplay of pro-angiogenic growth factors, IsoPs and the role of the TP receptors have been investigated thoroughly in different primary human endothelial cells. Low concentrations of 15-F2t-IsoP promoted endothelial cell migration. In contrast, higher concentrations of several E-, A- and F-series IsoPs inhibited the VEGF-induced migration and tube formation of endothelial cells. These effects were abolished either by TP receptors blockade or alternatively by short hairpin RNA-mediated knock down of the TP receptors.

Taken together, these findings highlight the role of 15-F2t-Isop but also of other IsoPs in vascular homeostasis and there by provide a new rationale for TP receptors blockade (Benndorf et al., 2008).

The retina is enriched with long chains PUFAs and is constantly exposed to light, rendering it highly vulnerable to OS. Because OS plays a key role in the pathogenesis of ocular neuropathies such as glaucoma and triggers spontaneous generation of long chain PUFA metabolites in retina, it is significant to delineate effect of these novel compounds on retinal pharmacology. So far, the pharmacological role for the 15-F2t-IsoP on neurotransmission in mammalian ocular tissues is well documented. However, the effect of the 5-F2-IsoP-series on ocular tissues has not been described. In a recent study, we elucidated the pharmacological actions, in vitro of the 5-F2t-IsoP epimer pair, 5-epi-5-F2t-IsoP (C5-OH in R-position) and 5-F2t-IsoP (C5-OH in S-position) on excitatory glutamate release (using [3H]D-aspartate as a marker) in bovine retina (Hou et al., 2004; Jamil et al., 2012; Zhao et al., 2009). Whereas 5-epi-5-F2t-IsoP elicited a concentration-dependent inhibitory action, the 5-(S)-OH-epimer, 5-F2t-IsoP displayed a more potent, biphasic inhibitory action on the neurotransmitter release, suggesting that spatial side chain orientation at the C5-position is required for the biphasic response. Consistent with the later observation, a biphasic profile of activity has been reported for 15-F2t-IsoP on the regulation of sympathetic and excitatory neurotransmission in the mammalian anterior uvea and retina, respectively. Contrary to 5-F2t-IsoP, the 15-F2t-IsoP lacks the hydroxyl side chain at C5 position. It is therefore apparent that additional factors contribute to the biphasic pattern of Iso-P-response on neurotransmitter release. Because the effect of their 15-F2t-IsoP-counterparts is largely dependent on activation of prostanoid receptors, Jamil et al. (2012) examined the role of prostanoid receptors in the inhibitory action of the 5-epi-5-F2t-IsoP. The inhibitory action of this 5-F2t-IsoP was reversed by the prostanoid EP1- (SC-51322; SC-19220) and EP4-(AH 23848) receptor antagonists but not the EP1–3/DP- (AH 6809) and DP/TP receptor antagonist (BAY-u3405). Due to the prominent role that glutamate plays in the physiology of the retina as the major excitatory neurotransmitter and in neuronal excitotoxicity, the ability of 5-F2t-IsoP to attenuate excitatory neurotransmitter release could have significant pathophysiological implications in mammalian retina. It is conceivable that these endogenously derived AA-metabolites could modulate progression of ocular neuropathies and provide a new target for diagnostic and/or therapeutic strategies in the management of ocular neuropathies. Taken together, these data support a modulatory role for 5-F2t-IsoP epimer pair, 5-epi-5-F2t-IsoP and 5-F2t-IsoP on excitatory neurotransmitter release in bovine retina, in vitro. Whereas the allylic hydroxyl group at position C5 contributes to the apparent biphasic pattern of response exhibited by 5-F2t-IsoP, the prostanoid EP1 and EP4 account for its inhibitory effect on excitatory neurotransmitter release (Opere et al., 2005).

### 4.2 Neuroprostanes

There is considerable evidence that an enriched n-3 PUFA diet confers cardioprotective effects due primarily to the two main PUFAs, EPA and DHA (GISSI-Prevenzione Investigators, 1999; Judé et al., 2003). This large prospective study showed that the most marked effect of DHA and EPA supplementation is a reduction of sudden cardiac death in the months following a cardiac infarction. This benefit has been explained, in part, by a reduction in arrhythmias and systolic cardiac failure. The anti-arrhythmic effects of n-3 PUFAs have been confirmed in animal models of cardiac infarction by ligature of the left coronary artery. These and other studies in single cardiac cells have shown that EPA and DHA can modulate the activity of ion channels, the transmembrane proteins responsible for the electrical activity of the heart. However, it has been suggested that oxygenated metabolites of EPA and DHA may also play a role in these actions. In this regard it has been shown that some of the effect of DHA on rat cardiac ion channels was due to an oxidative metabolite of DHA (Judé et al., 2003).

Le Guennec et al. tested different F2-IsoP, F3-IsoP and F4-NeuroP on arrhythmias induced by isoprenaline and stimulation frequency of isolated ventricular mice cardiac cells. Among them, some F4-NeuroPs have anti-arrhythmic properties (AAP) ($IC_{50} \approx 100$ nM) (Le Guennec, 5 December 2012). The main isomer, 4(RS)-4-F4t-NeuroP (Fig. 9), showed potent...
dose-dependent AAP in cellulo and also in vivo in PM1 mice. At the cellular level, the mechanism of action is unlikely to be due to a β-blocker effect; but the AAP can instead be explained by a rycal-like effect; in particular, stabilization of the RyR2 complex with FKBP12.6 (Andersson and Marks, 2010; Roy, 2015).

4.3 Phytoprostanes

PhytoPs formed in higher plants from ALA, are highly active lipids in plant kingdom and in humans. Experimental evidence strongly suggests that in plants PhytoPs act as endogenous mediators able to protect cells from damages under various conditions (Conconi et al., 1996, Thoma et al., 2003), especially those related to OS (Loeffler et al., 2005). Since humans are potentially exposed to PhytoPs, which can be absorbed after oral ingestion of vegetable food or by inhalation of pollen (Gilles et al., 2009), it is important to identify their activities in animal cells (Gutermuth et al., 2007).

Minghetti et al. (2014) investigated the possible effect of 9-L1-PhytoP (Fig. 10) on cells of the central nervous system (CNS), which are particularly susceptible to OS. Two experimental models were used: the SH-S5Y5 cell line, from a human neuroblastoma as a neuronal model and primary cultures of oligodendrocyte progenitors (OLs) from neonatal rat brain.

9-L1-PhytoP can exert protective effects on cells in CNS, in particular, they protect neuronal cells not yet differentiated, such as those found in the adult brain from damages related to OS. They also promote the differentiation of OLs, through mechanisms which involve in part the activation of the nuclear receptor and ligand dependent transcription factor PPARγ.

5 Conclusions

Our understanding of the role of PUFA peroxidation in the pathogenesis of various human diseases is at an early stage. We know that free radical-induced autoxidation of PUFA occurs in numerous pathological conditions from cardiovascular disorders to cancers and neurodegenerative diseases. Early work with animal tissues indicated that ROS, or radicals formed after exposure to oxidants, are the initial triggers for oxidative injury to membranes. Subsequently, it was shown that IsoP generation accompanied lipid peroxidation injury in mammalian tissues. The exact PUFA derivatives generated will depend on the fatty acid composition of the tissue, an important consideration for the use of IsoPs or NeuroPs as an organ-specific biomarker. Plant cells subjected to OS also produce cyclopentenone prostanooids, termed PhytoPs, by a non-enzymatic mechanism. Through our knowledge of organic chemistry, we can contribute to clinical and basic research by developing novel synthetic approaches and providing samples for biological and analytical work. A number of new approaches for chiral synthesis of IsoPs, PhytoPs NeuroPs are now available. Some of these products may be used as markers for the diagnosis and management of patients and will need to be measured accurately and precisely. The contribution of each of these unique PUFA derivatives to tissue and organ damage has to be clearly ascertained within a complex network of signalling molecules and mediators.

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References


