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Isoprostanes and neuroprostanes: Total synthesis, biological activity and biomarkers of oxidative stress in humans

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ABSTRACT

Isoprostanes (IsoPs) and neuroprostanes (NeuroPs) are formed *in vivo* by a free radical non-enzymatic mechanism involving peroxidation of arachidonic acid (AA, C20:4 n-6) and docosahexaenoic acid (DHA, C22:6 n-3) respectively. This review summarises our research in the total synthesis of these lipid metabolites, as well as their biological activities and their utility as biomarkers of oxidative stress in humans.

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1. Introduction

Free radicals have been implicated in a wide variety of human disorders [1] and are known to oxidize biomolecules, including DNA, proteins and lipids. Polyunsaturated fatty acids (PUFAs) are unstable lipids, due to the presence of multiple double bonds that are subject to react with free radicals to form numerous oxygenated metabolites [2]. There has been considerable research in isoprostanes (IsoPs) [2] since their discovery by Morrow et al. in 1990 [3]. The F₂-IsoPs are formed *in vivo* predominantly by free radical non-enzymatic oxidation of arachidonic acid (AA, C20:4 n-6), although there is some evidence to suggest F₂-IsoPs can be derived, in part, *via* a cyclooxygenase-induced pathway [4]. There are numerous reports demonstrating IsoPs are the most reliable biomarkers of oxidative stress *in vitro* and in animal models [5], as well as in humans [6]. Additionally, several IsoPs have also been shown to be biologically active [2].

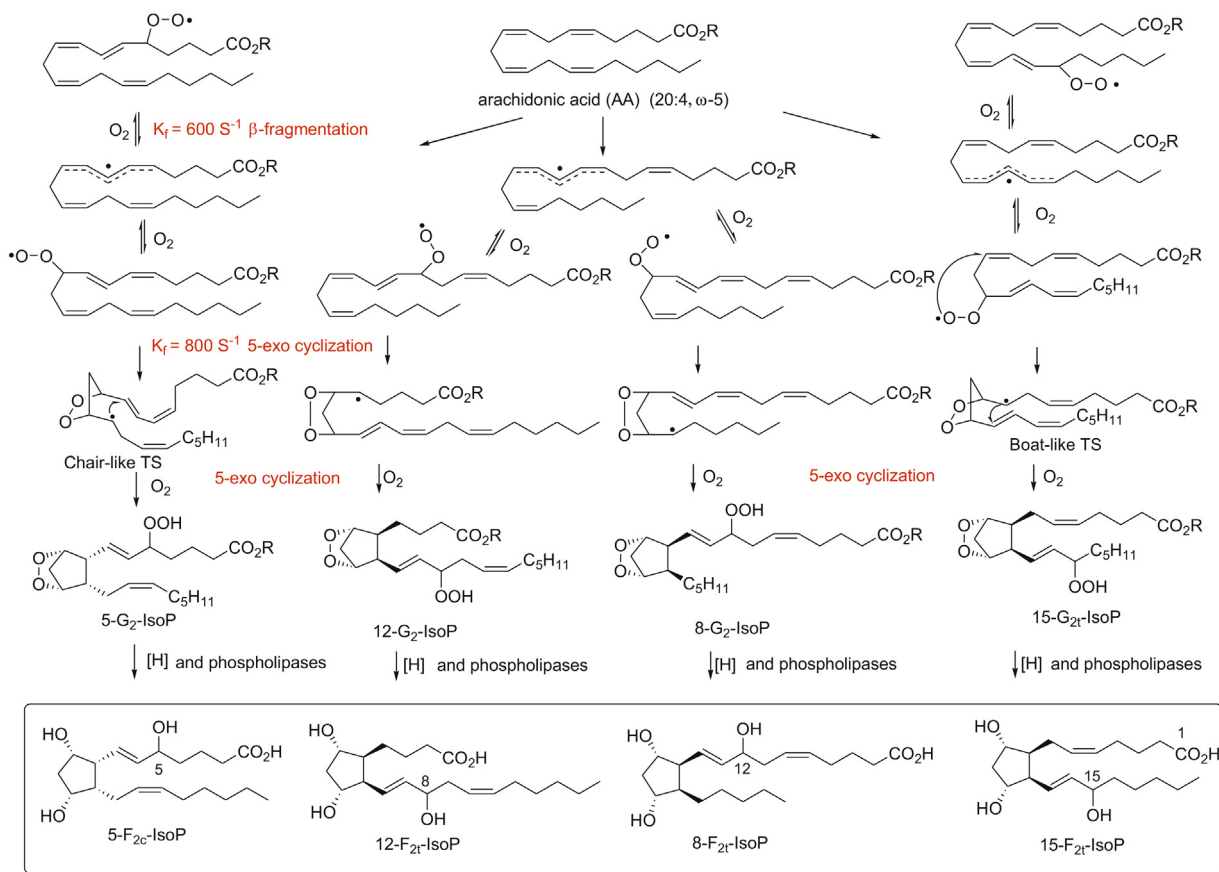
Subsequent to the reporting of F₂-IsoPs, others have described oxidation products of the n-3 fatty acids alpha-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), yields the phytoprostanes [7], F₃-IsoPs [8] and F₄-IsoPs or neuroprostanes (NeuroPs) [9], respectively. More recently, dihomo-isoprostanes (Dihomo-IsoPs) derived from adrenic acid (AdA, C22:4 n-6) have been reported [10]. DHA is located mainly in brain grey matter and AdA in brain white matter. Other oxidative metabolites of these and other fatty acids, including A-, D-, E- and J-IsoPs, have been described in the literature [2]. More recently, the isofurans (IsoFs), formed from free radical-induced peroxidation of AA but under conditions of high oxygen tension, have been described [11,12].

This review describes strategies for the total synthesis of E-, D- and F-IsoPs, NeuroPs and Dihomo-IsoPs. It will focus on those IsoPs and NeuroPs that have been found *in vivo*, including their physiological activity and utility as biomarkers of oxidative stress in humans.

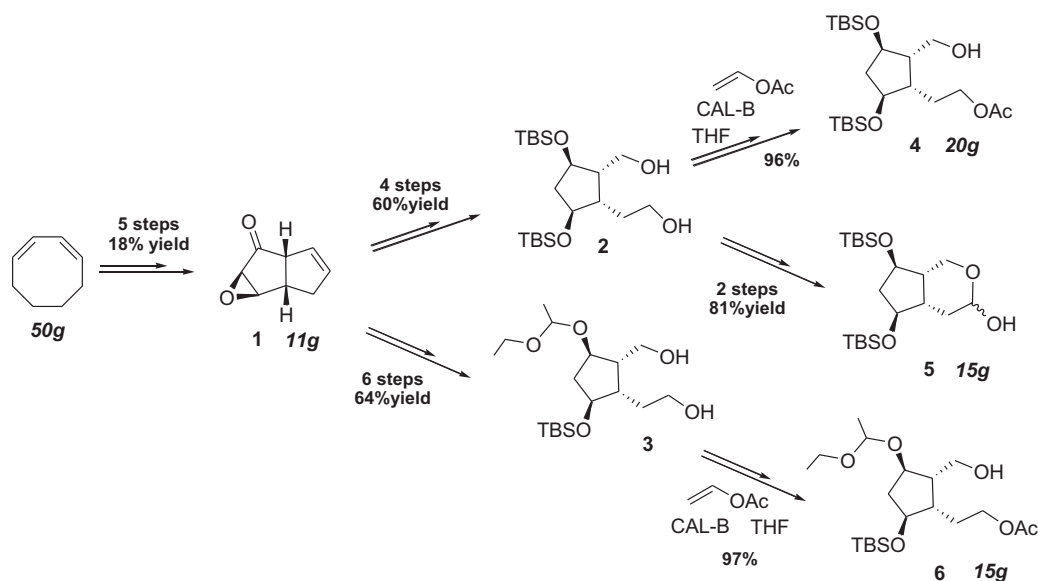
2. Biosynthesis

The biosynthesis of F-IsoPs (at the time referred as PG-like compounds) was first described in the mid 70s while research was being carried out into the elucidation of the biosynthesis of prostaglandins [13,14]. Subsequent to this, Roberts, Morrow and co-workers in 1990 [3], proposed a pathway to account for the non-enzymatic peroxidation of arachidonic acid bound to phospholipids, leading to novel PG-like compounds which they named Isoprostanes (IsoPs) [5,15]. The F-IsoPs are released as free acids by the platelet-activating factor acetylhydrolase and possibly other phospholipases [16,17], circulate predominantly in high density lipoproteins [18] in plasma, and are excreted in urine where a significant proportion of F₂-IsoPs are conjugated as glucuronides [19].

The pathway for IsoP synthesis is initiated by hydrogen abstraction at one of the bis-allylic positions of the corresponding PUFA (Scheme 1). The transient pentadienyl radical is oxygenated at its terminal position to give pentadienyl peroxy radicals. This oxygenated radical can have several fates leading to a number of metabolites, one of them involves irreversible O-C/C-C bicyclization (double 5-exo-trig cyclization) to available double bonds, followed by addition of oxygen and H-transfer yielding G-type IsoPs. Reduction of the hydroperoxide group is followed by the



Scheme 1. Isoprostanes (IsoPs) formation from arachidonic acid (AA).



Scheme 2. New strategy towards the synthesis of isoprostanes, neuroprostanes.

non-enzymatic reduction or rearrangement of the endoperoxide moiety (to the contrary of cell specific PG-synthases). F-type IsoPs are generated under normal condition while, E- and D-type arise from the known Kornblum–DeLaMare rearrangement [20] in aqueous basic media. Dehydration of membrane-bound E₂- and D₂-IsoPs, is facile under physiological conditions and produces cyclopentenone-A₂- and -J₂-IsoPs respectively, *in vitro* and *in vivo*.

Of particular importance is the *cis* orientation of the side chains in IsoPs to the contrary of the *trans* orientation in PGs. This difference reflects the biosynthesis of IsoPs that follows conventional chemistry rules (lower transition state energy during the double 5-exo-trig cyclization) compared to enzymatically driven three-dimensional orientation for PG synthesis. Furthermore, two different stereochemistries are present in IsoPs, the all-*syn* (represented as subscript “c”; see 5-F_{2c}-IsoP) and *syn-anti-syn* stereochemistry (represented as subscript “t”; see 15-F_{2t}-IsoP) again depending of the two lower transition states possible during cyclization (chair- and boat-like transition states are shown in Scheme 1). Theoretically, there are four F₂-IsoPs regioisomers each with 8 racemic diastereoisomers, generating 64 possible compounds. Waugh et al. [21] and later Li et al. [22] showed from *in vitro* and *in vivo* studies that the 5- and 15-series IsoPs are formed in significantly greater amounts than the 8- and 12-series IsoPs. Current evidence suggests that the 5- and 15-series IsoPs are most abundant *in vivo*, due to the fact that the 8- and 12-series IsoPs are more readily metabolised [23].

Oxidation of DHA by similar mechanisms to that of arachidonic acid (Scheme 1) yields 8 possible regioisomers termed 4-, 7-, 10-, 11-, 13-, 14-, 17- and 20-series NeuroPs, and theoretically, a total of 128 compounds. Yin et al. [24] provided experimental evidence that the 4- and 20-series NeuroPs are the two most abundant NeuroP regioisomers generated from the autoxidation of DHA both *in vitro* and *in vivo*. VanRollins et al. [10] described AdA oxidation yields four series of regioisomeric isoprostanoids termed 7-, 10-, 14-, and 17-dihomo-IsoPs with the 7- and 17-series being the most abundant.

3. Chemical synthesis

In order to fully assess the physiological importance of each of the enantiomerically pure IsoPs, NeuroPs and dihom-IsoPs, we have developed different chemical strategies [2]. Since 1990, three

strategies have been developed by Durand’s group, based on radical carbocyclization [25], furan ring transformation [26], and the last utilizing a bicyclo[3.3.0]octene intermediate [27]. In this review, we will focus on our most recent strategy and on the total syntheses of IsoPs, NeuroPs and dihom-IsoPs.

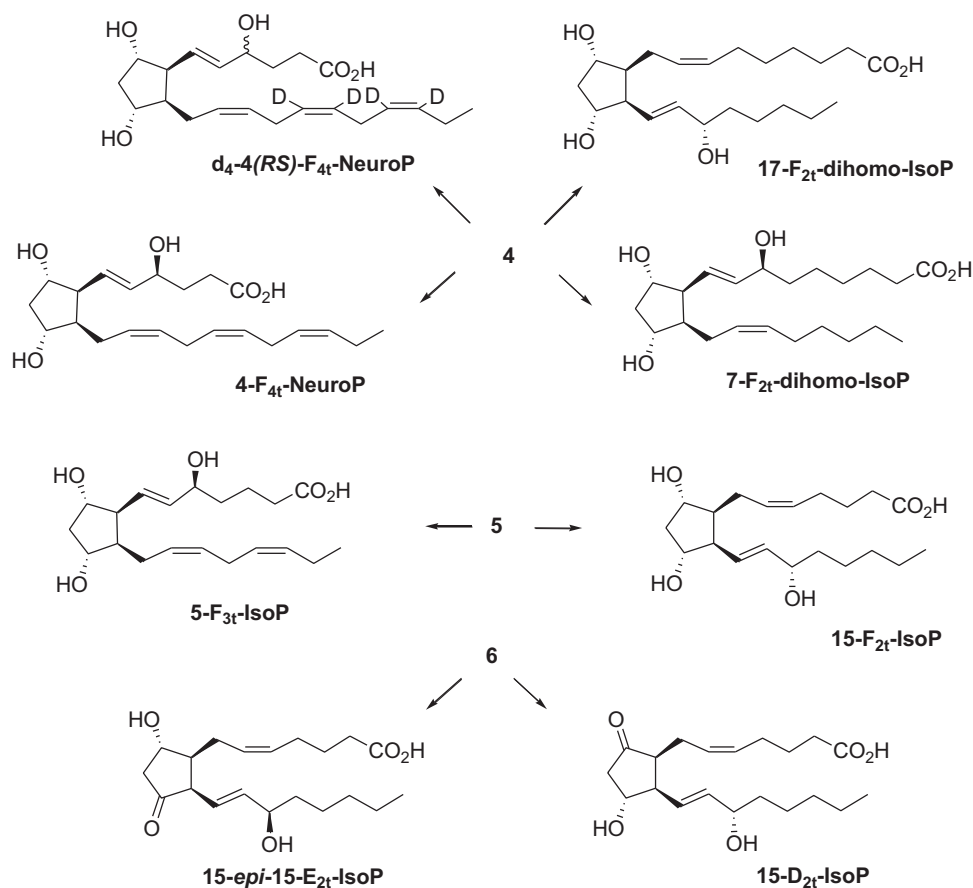
This strategy uses a bicyclo[3.3.0]octene scaffold (1) and focuses on E-, D-, F-IsoPs with *syn-anti-syn* stereochemistry [27]. Bicyclo[3.3.0]octene intermediate 1 is readily obtained from 1,3-cyclooctadiene in 5 steps (18% yield). The two enantiomers are obtained using enzymatic resolution. Bicyclo[3.3.0]octene 1 is transformed into 1,5-diols 2 and 3 in several steps. In order to access E- and D-IsoPs, this strategy provides an orthogonal protection of the 1,3-*cis*-diol functionality (see compound 3), allowing at a later stage of the synthesis a selective deprotection of one of the two protected hydroxyls, when compound 2 allowed the synthesis of F-IsoPs. With the *syn-anti-syn* stereochemistry introduced, the subsequent steps of the synthesis involve introduction of the side chains and desymmetrisation of the two hydroxyl groups. This strategy allows diol 2 to be either selectively oxidized into lactol 5 or selectively and enzymatically protected into monoacetate 4 [28]. In the same way diol 3 is selectively protected in high yield into monoacetate 6 (Scheme 2).

The synthesis of E-, D-, F-IsoPs, NeuroPs or dihom-IsoPs is achieved using the three synthetically advanced intermediates (4, 5 and 6) (Scheme 2). Lateral chains are introduced using Wittig, Horner–Wadsworth–Emmons or cross metathesis methodologies. Depending on the nature of the coupling reagent (phosphonium salt, α -ketophosphonate), one intermediate is preferred and allows a flexibility in the synthesis.

We have synthesized a number of E- and D- [29], and F-series IsoPs, as well as NeuroPs [30] and dihom-IsoPs [31] using this new methodology (Scheme 3).

4. Biomarkers of lipid peroxidation

Quantification of products of oxidative damage in biological systems is important in order to understand the role of free radicals in disease states [32]. Lipids that undergo peroxidation, represent major targets of free radical attack. F₂-IsoPs are considered to represent the most reliable marker of *in vivo* lipid peroxidation and oxidative stress [5,33]. F₂-IsoPs are stable oxidation products of lipid peroxidation [34]. Although there is some evidence



Scheme 3. Total synthesis of isoprostanes, dihydroisoprostanes and neuroprostanes.

that F₂-IsoPs may, in part, be formed *via* a cyclooxygenase (COX)-dependent pathway, this appears to be dependent upon a number of factors [35]. In humans McAdam et al. [36] showed that urinary F₂-IsoPs were formed independent of COX-1 and COX-2. Similarly, Bachi et al. [37] showed that in humans, but not in rats, urinary F₂-IsoPs were formed independent of COX-1. In contrast, *in vitro* studies showed F₂-IsoPs were increased in J774 macrophages with COX-2 induction [38]. However, F₂-IsoPs were not inhibited by COX-1 or COX-2 inhibition in human isolated pulmonary artery smooth muscle cells [39].

The measurement of F₂-IsoPs with gas chromatography-mass spectrometry (GCMS) using electron capture negative ionization is considered the “gold standard”. It is important to note that although F₂-IsoPs can be measured by enzyme-linked immunoassay [40,41] we have shown poor agreement between mass spectrometry and enzyme-linked immunoassay [42].

The information gained from measurement of different lipid peroxidation markers depends on the clinical situation and therefore the choice of markers should be carefully considered. In the following discussion we present examples from our research where the measurements of IsoPs, IsoFs and NeuroPs have been used in clinical trials to elucidate the role of oxidative stress in clinical situations.

4.1. Effects of type of anaesthesia and oxygen concentration during surgery

Ischemia/reperfusion injury (IRI) is one of the main pathological phenomena observed in orthopaedic surgery. The application and release of a tourniquet is often used in elective total knee replacement surgery to reduce blood loss and obtain

a clearer surgical field. IRI, in which oxidative injury plays a fundamental role, results in a local and systemic inflammatory response. Surgery utilises two anaesthetic techniques: spinal anaesthesia (SA) or general anaesthesia (GA), where the levels of inspired oxygen can differ. There is also evidence that spinal anaesthesia (SA) reduces the risk of postoperative mortality and morbidity [43] with a reduction of postoperative vascular events. In a randomized blinded study we examined the effects of SA and GA on markers of oxidative stress (plasma F₂-IsoPs and IsoFs) in patients undergoing knee replacement surgery. F₂-IsoPs were significantly lower in the GA patients compared with SA patients. In contrast, the GA patients had significantly elevated plasma IsoFs. Increased IsoFs during GA compared with SA likely reflect increased oxidative stress due to elevated oxygen concentrations during GA. Under conditions of higher oxygen intake such as GA the balance of arachidonic acid metabolism by free radicals is shifted from F₂-IsoPs to IsoFs formation [44]. In a subsequent study, we examined the effect of altering inspired oxygen concentrations in patients undergoing ischemia/reperfusion during upper arm surgery [45]. We showed plasma IsoFs were positively associated with oxygen tension (PvO₂) and this relationship was significantly attenuated by blood hemoglobin concentration. This is noteworthy given that hemoglobin *per se* did not significantly affect plasma IsoFs. Plasma F₂-IsoP during reperfusion was also not different between the groups and there was no significant relationship between F₂-IsoP and PvO₂ or hemoglobin concentration.

4.2. Brain injury

The high oxygen requirements of the brain for metabolism and its high polyunsaturated fatty acid composition, in particular

DHA, make the brain vulnerable to oxidative insult. F₄-NeuroPs are considered markers of brain related oxidative stress [46]. Aneurysmal subarachnoid hemorrhage (aSAH) and traumatic brain injury (TBI) are associated with devastating central nervous system (CNS) injury. Acute brain injury, is thought to associate with overproduction of reactive oxygen species (ROS). In two case-controlled studies [47] 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 F₄-NeuroPs and F₂-IsoPs. Patients with TBI had significantly increased CSF F₄-NeuroPs but F₂-IsoPs were not different from their controls. These data confirm that CNS injury as a result of aSAH or TBI results in increased oxidative stress. Since DHA is the major polyunsaturated fatty acid in the brain, F₄-NeuroP levels in CSF may be a more specific indicator of possible neurological dysfunction than F₂-IsoPs. Hsieh et al. [48] showed that increased F₄-NeuroPs in CSF of patients with aSAH correlated with poor neurological outcome and suggested that F₄-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. [49] showed increased F₂-isoPs during rat brain ischemia, the E₂/D₂-IsoPs were increased to a greater extent, suggesting the latter may be better markers of oxidative stress in brain ischemia.

4.3. Pre-eclampsia

Pre-eclampsia is a life-threatening disorder of pregnancy that adversely affects the mother and the baby. Oxidative stress may contribute to the pathogenesis of this syndrome. Previously, we have shown that plasma F₂-IsoP are raised in proteinuric pre-eclampsia [50]. In a recent case-controlled study [51] we examined IsoFs, F₄-NeuroPs and F₂-IsoPs in maternal plasma and cord blood of women with pre-eclampsia and normal pregnancies. Women with pre-eclampsia had significantly elevated maternal IsoFs and F₄-NeuroPs, but not F₂-IsoPs. Cord blood F₄-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 and could reflect the oxidative challenge presented at birth, when there is transition from a relatively low intrauterine oxygen environment to a significantly higher extrauterine oxygen environment. We also found maternal F₄-NeuroPs were not significantly correlated with cord blood F₄-NeuroPs in either pre-eclamptic or normal pregnancies, suggesting the origin of cord F₄-NeuroPs may be independent of maternal plasma. In normal pregnancy birth weight was negatively related to maternal F₂-IsoPs, IsoFs and F₄-NeuroPs.

4.4. Fish oil supplementation

In two placebo-controlled interventions in (1) overweight, dyslipidaemic men; and (2) treated-hypertensive Type 2 diabetic patients, randomized to daily EPA, or DHA or placebo, we showed post-intervention plasma and urinary F₂-IsoPs were significantly reduced by EPA and by DHA [52,53]. Neither F₃-IsoPs – nor F₄-NeuroPs were observed in plasma in both studies. These findings support our previous reports that have shown n-3 fatty acids reduce oxidative stress, in part, via attenuation of inflammation.

4.5. Rett syndrome

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) gene [54]. 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 [55]. 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 [56]. Clinical evidence indicates that F₂-IsoPs and F₄-NeuroPs are involved in the intimate pathogenetic mechanisms of RTT. Plasma levels of free F₂-IsoPs are significantly higher in the early stages of RTT, as compared with the late natural progression of typical RTT [57].

F₂-dihomo-IsoPs are significantly increased in RTT [58]. Due to the relative abundance in myelin of the precursor fatty acid [10,59] the increased formation of F₂-dihomo-IsoPs, particularly in the early stages of the disease, strongly suggests the coexistence of an early damage to the brain white matter. Until recently it was thought that the predominant central nervous system damage in RTT occurred in gray matter. However, our data [58] have contributed to generate the hypothesis that early brain white matter damage may represent an early event in RTT as suggested by previous brain MRI evidence [60]. Thus F₂-dihomo-IsoPs can be considered early markers of lipid peroxidation in RTT.

F₄-NeuroPs also appear to be an important biomarker of RTT [61]. Plasma F₄-NeuroPs correlate with disease severity in RTT [61] and are significantly related to neurological symptoms severity, mutation type and clinical presentation [61]. Therefore, F₄-NeuroPs may play a major role along the biochemical pathway from MeCP2 gene mutation to the disease clinical presentation, thus testifying that a DHA oxidation process is occurring.

5. Bioactive lipids

Isoprostanes are not only biomarkers of lipid peroxidation but also mediators of oxidant injury. They are vasoconstrictors in many species and various vascular beds (reviewed in Ref. [62]), modulate platelet activity (reviewed in Ref. [63]) and monocyte adhesion [64,65], and induce proliferation of endothelial and smooth muscle cells [66,67]. Isoprostanes mediate their biological effects by activation and/or inhibition of several prostanoid receptors, among them the thromboxane receptor (TP), prostaglandin F_{2α} receptor (FP), prostaglandin E₂ subtype 3 receptor (EP3), prostaglandin D₂ subtype 2 receptor (DP2) and by activation of the peroxisome proliferators activated receptor gamma (PPARγ) [68–72].

5.1. Mammalian vascular tissues

The vasomotor action of 15-F_{2t}-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 [69,73–78]. In contrast to 15-F_{2t}-IsoP, 5-F₂-IsoP-series do not contribute to the vasoconstriction mediated by isoprostanes [79]. Besides vasoconstriction and platelet activation, isoprostanes also enhance the vascular reperfusion damage after myocardial infarction [80]; pioneering cardiac smooth muscle apoptosis and scar formation. In this scenario, formation of collaterals 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 has been investigated thoroughly in different primary human endothelial cells [81]. Low concentrations of 15-F_{2t}-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 blockade or alternatively by short hairpin RNA-mediated knock down of

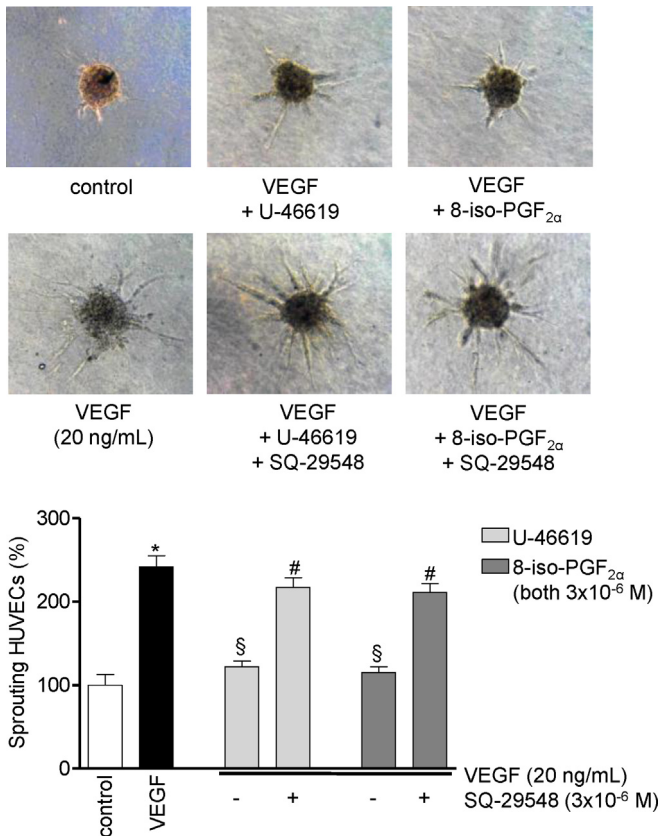


Fig. 1. Influence of 8-iso-PGF_{2α} (15-F_{2t}-IsoP) on VEGF-induced sprouting of endothelial cells. The thromboxane A₂ receptor agonists U-46619 and 8-iso-PGF_{2α} (15-F_{2t}-IsoP) both 3×10^{-6} M inhibit the VEGF (20 ng/mL)-induced sprouting of HUVECs (U-46619 $122 \pm 7\%$, 8-iso-PGF_{2α} $115 \pm 7\%$, § $p < 0.001$ vs. VEGF $242 \pm 14\%$). This effect is blocked through the thromboxane A₂ receptor antagonist SQ-29548 (3×10^{-6} M; U-46619 + SQ-29548 $217 \pm 12\%$, 8-iso-PGF_{2α} + SQ-29548 $211 \pm 10\%$, # $p < 0.001$ vs. U-46619/8-iso-PGF_{2α}).

the TP. Taken together, these findings highlight the role of 15-F_{2t}-IsoP but also of other IsoPs in vascular homeostasis and thereby provide a new rationale for TP blockade (Fig. 1).

5.2. Mammalian retina

The retina is enriched with LCPUFAs and is constantly exposed to light, rendering it highly vulnerable to oxidant stress [82]. Because oxidant stress plays a key role in the pathogenesis of ocular neuropathies such as glaucoma [83] and triggers spontaneous generation of LCPUFA metabolites in retina [84], it is significant to delineate effect of these novel compounds on retinal pharmacology. So far, the pharmacological role for the 15-F₂-IsoPs on neurotransmission in mammalian ocular tissues is well documented [84]. 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 of the 5-F₂-IsoP epimer pair, 5-*epi*-5-F_{2t}-IsoP (C5-OH in α -position) and 5-F_{2t}-IsoP (C5-OH in β -position) on excitatory glutamate release (using [³H]D-aspartate as a marker) in bovine retina, *in vitro* [85]. Whereas 5-*epi*-5-F_{2t}-IsoP elicited a concentration-dependent inhibitory action, the 5-(S)-OH-epimer, 5-F_{2t}-IsoP displayed a more potent, biphasic inhibitory action on the neurotransmitter release [85], suggesting that spatial side chain orientation at the C5-position is accounts for the biphasic response. Consistent with the later observation, a biphasic profile of activity been reported for 15-F_{2t}-IsoP on the regulation of sympathetic and excitatory neurotransmission in the mammalian anterior uvea

and retina, respectively [84]. Contrary to 5-F_{2t}-IsoP, the 15-F_{2t}-IsoP lacks the hydroxyl side chain at C5 position. It is therefore apparent that additional factors contribute to the biphasic pattern of IsoP-response on neurotransmitter release.

Because the effect of their 15-F₂-IsoP-counterparts are largely dependent on activation of prostanoid receptors, Jamil et al. [85] examined the role of prostanoid receptors in the inhibitory action of the 5-*epi*-5-F_{2t}-IsoP. The inhibitory action of this 5-F₂-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-F₂-IsoPs 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 [85]. Taken together, these data support a modulatory role for 5-F₂-IsoP epimer pair, 5-*epi*-5-F_{2t}-IsoP and 5-F_{2t}-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-F_{2t}-IsoP, the prostanoid EP1 and EP4 account for its inhibitory effect on excitatory neurotransmitter release.

5.3. Anti-arrhythmic activities

There is considerable evidence that a diet enriched n-3 PUFAs confers cardioprotective effects due primarily to the two main PUFAs EPA and DHA [86]. A 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 [87]. 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 ligation of the left coronary artery [88]. 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 [89]. However, it has been suggested that oxygenated metabolites of EPA and DHA may also play a role in these actions [88]. In this regard it has been shown that some of the effect of DHA on rat cardiac ion channels is due to an oxidative metabolite of DHA [90]. Le Guennec et al. [91] tested different F₂-IsoPs, F₃-IsoPs and F₄-NeuroPs on arrhythmias induced by isoprenaline and stimulation frequency of isolated ventricular mice cardiac cells. Among them, some F₄-NeuroPs have anti-arrhythmic effects (IC₅₀ \approx 100 nM).

6. Outlooks and conclusions

Our understanding of the role of PUFA peroxidation in the pathogenesis of various diseases continues to expand. The discovery and study of IsoPs have provided a major step forward in the field of free radical research. A number of IsoPs and NeuroPs have been synthesised allowing researchers to examine their biological activities and evaluate their potential role as markers of oxidative damage in a number of clinical and experimental studies. IsoPs, IsoFs and NeuroPs measured by mass spectrometry can be useful in elucidating the role of oxidative stress in the clinical setting. Further studies are required to determine how these markers of oxidative stress relate to severity of complications and clinical outcomes.

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