Cardiovascular pharmacology and physiology of the isoprostanes

Jean-Luc Cracowski*a, Thierry Durandb

aLaboratoire de Pharmacologie, Inserm ESPRI, HP2 EA 3745, Faculté de Médecine de Grenoble, France
bUMR CNRS, 5074, Faculté de Pharmacie, Université Montpellier I, Montpellier, France

INTRODUCTION

Isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism. Their quantification as a pathophysiological biomarker provides a unique opportunity to investigate lipid peroxidation in vascular diseases. Their measurement also provides an interesting biomarker for the rational dose selection of antioxidants in vascular diseases where oxidative stress might be involved. In addition to their use as biomarkers, some isoprostanes possess a biological activity. The 15-series F2* and E2-isoprostanes mediate vasoconstriction in different vascular beds and species. In addition, 15-F2t-IsoP induces smooth muscle cells mitogenesis and monocyte adhesion to endothelial cells. The data available supports but does not prove the hypothesis that isoprostanes are involved in vascular physiology and pathogenesis.

ISOPROSTANE STRUCTURE AND SYNTHESIS

Depending on which of the labile hydrogen atoms of arachidonic acid is first abstracted by free radicals, three initial arachidonoyl radicals can be formed following free radical attack. These radicals form four prostaglandin-H1-like compounds that can then be fully reduced to form four prostaglandin F2α regioisomers (Figure 1), or rearranged to form prostaglandin E2 and D2 regioisomers. Two mechanisms, based on the formation of a 'dioxetane' intermediate, via a 4-exo-cyclization or a β-fragmentation followed by successive 5-exo-cyclizations have been proposed recently for the formation of these compounds [4]. Because each F2-isoprostane regioisomer comprises eight diastereoisomers, 64 different F2-isoprostanes can be generated.

Isoprostanes were formerly named according to the prostaglandin F2α chemical structure. They differ from prostaglandins by the cis-stereochemistry of the five-membered ring junction instead of the trans-stereochemistry of the prostaglandin F2α. Because the first isoprostanes described were the 15-series, they were formerly named according to this major difference, the first isoprostane being named 8-iso-prostaglandin F2α or 8-epi-prostaglandin F2α. However, such a nomenclature does not allow the differentiation of the numerous isomeric structures. Two nomenclatures were proposed.
recently, both of which enable an easy differentiation of the isoprostane isomers. Taber et al. [5] nomenclature was filed with the Eicosanoid Nomenclature Committee, and approved by the International Union of Pure and Applied Chemistry. Rokach et al. [6] also proposed a nomenclature that enables the differentiation of the regioisomers. In 1997, this nomenclature was modified to be applicable to isoprostane-like compounds derived from eicosapentaenoic and docosahexaenoic acid. The concomitant use of these three different nomenclatures is confusing for the nonspecialist, and we propose that the old nomenclature (e.g. 8-iso-prostaglandin F2a) be definitively abandoned, and that Taber’s nomenclature be encouraged. The different nomenclatures used to name the main isoprostanes are given in Figure 2.

Several in vitro studies have suggested a cyclooxygenase (COX)-dependent formation of 15-F2t-IsoP [7–9]. An efficient in vivo production of 15-F2t-IsoP through the COX pathway would reduce its accuracy as a valid marker of lipid peroxidation. In contrast to the in vitro data, clinical studies clearly showed that COX inhibition was unable to decrease the formation of F2t-isoprostanes in healthy subjects as well as patients, suggesting that F2t-isoprostanes are formed via a non-COX-dependent mechanism in vivo [10–14]. Furthermore, in conditions of increased COX-2 expression following intravenous lipopolysaccharide challenge, the formation of 15-F2t-IsoP and of 5-series isomers was not altered by COX inhibitors in healthy volunteers, whereas prostanoid production was decreased, further suggesting a COX-independent pathway of F2t-isoprostane synthesis [15]. Finally, in vivo COX-dependent formation of IPF2-III has been shown in the rat but not in humans [16]. Altogether, these data suggest that although a COX-dependent formation can be demonstrated in vitro, this does not occur in vivo in humans, meaning that 15-F2t-IsoP as well as the 5-series isomers can be used as biomarkers of lipid peroxidation in vivo.
ISOPROSTANE QUANTIFICATION

Quantification of F2-isoprostanes is used as a reliable marker of lipid peroxidation in vivo [17], and several methods are currently used [18] including gas chromatography (GC)–mass spectrometry (MS), which might be associated with an immunoaffinity extraction, GC–tandem MS, and liquid chromatography–tandem MS. These methods are reviewed in detail elsewhere [19]. They are specific but their cost and technology limit their routine use. Measurement of urinary 15-F2t-IsoP by radioimmunoassay has been validated and constitutes a valid and easier alternative to GC–MS [20]. Enzyme immunoassays have also been developed to measure levels of F2-isoprostanes but the antibodies used have not been tested for cross-reactivity with the numerous F2-isoprostane isomers and their metabolites. The results obtained using enzyme immunoassays sometimes differ from those obtained using GC–MS assays and therefore immunoassays should be considered as semi-quantitative indices of F2-isoprostanes.

VASCULAR PHARMACOLOGY OF THE ISOPROSTANES

Isoprostanes are formed in situ on phospholipids, at sites of free radical generation. Once released from cell membranes by phospholipases, isoprostanes circulate in the plasma in free forms and are therefore susceptible to activate membrane receptors. Most studies have focused on the biological activity of 15-F2t-IsoP, the first isoprostane commercially available. 15-F2t-IsoP is a vasoconstrictor in most species and vascular beds, both in vitro and in vivo, following intravenous administration (see [21,22] for full reviews). These constrictor properties are not specific to the blood vessels and have been demonstrated in the lymphatic vessels, the bronchi, the gastrointestinal tract and the uterus. In addition, it stimulates mitogenesis in uterine vascular smooth muscle cells [23]. The available data strongly suggest that the effects of 15-F2t-IsoP on blood vessels are mediated by the activation of the TP receptors (thromboxane A2/prostaglandin H2 receptors), acting as a full or partial agonist [24,25], although some responses including mitogenesis appear to be at least in part TP receptor independent. The existence of a specific isoprostane receptor has been suggested but remains to be elucidated [26]. In addition, preliminary data suggest that isoprostanes, as well as their precursor arachidonic acid, are other lipid ligands for the PPAR [27].

The effects of 15-F2t-IsoP on platelets are complex. When incubated with subthreshold concentrations of ADP, thrombin, collagen and arachidonic acid, 15-F2t-IsoP causes irreversible platelet aggregation, dependent
on thromboxane generation, while 15-F₂tısoP alone induces weak, reversible aggregation, only at high concentrations [28]. As 15-F₂tısoP is a partial agonist at the prostanoid TP receptor on platelets, it might inhibit the pro-aggregatory effects of TP receptor stimulation. Indeed, in human whole blood, 15-F₂tısoP is anti-aggregatory [29]. Several authors suggested that increased isoprostane formation is one of the factors involved in aspirin resistance, but a full demonstration of this hypothesis is not available to date [30–33].

15-F₂tısoP-induced contraction is modulated by the endothelium through the release of NO, i.e. endothelium removal increases 15-F₂tısoP contraction [21]. In addition, 15-F₂tısoP induces both thromboxane A₂ and endothelin-1 release from endothelial cells (Figure 3). In comparison with the huge data available for 15-F₂tısoP, few are available for other isomers. Nevertheless, other isoprostanes belonging to the 15 series of the F-family isoprostanes, such as 9-epi-15-F₂tısoP and 15-epi-15-F₂tısoP are biologically active, although less potent than 15-F₂tısoP [21]. The 5-series and 15-series F₂-isoprostanes are produced in approximately equal amounts in vivo whereas the 8-series and 12-series F₂-isoprostanes are produced in lower amounts [34]. In human urine and plasma, the 5-series (e.g. 5-F₂tısoP and 5-F₂tısoP) was found to be the most abundant F₂-isoprostanes [35]. Both the 15-series and the 5-series are easily detectable in human urine and plasma. However, in contrast to the 15-series F₂-isoprostanes, the 5-series F₂-isoprostanes has no vasomotor effect in different species and vascular beds [36] and as such are unlikely to be involved in the pathogenesis of vascular diseases. In addition to the vasoconstrictor and mitogenic effects, 15-F₂tısoP and 15-E₂tısoP induce monocyte adhesion to endothelial cells [37,38], whereas 15-F₂tısoP is a specific activator of rapid neutrophil adhesion [39]. Furthermore, 15-F₂tısoP induces cerebral endothelial cell death [40]. Endothelial cells are one of the first targets of oxidative stress in atherogenesis and ischemia–reperfusion injury. Whether isoprostanes may be one of pathogenic mediators remains to be tested.

Compared with the F₂-isoprostanes, E₂-isoprostanes are more potent in vitro. 15-E₂tısoP is more potent than 15-F₂tısoP in systemic and pulmonary vessels, its contraction being mediated through TP receptor, and EP₃ receptor activation in the pulmonary vasculature [41,42]. In addition, 15-E₂tısoP may induce a relaxation through EP receptors [43]. However, because no data are available concerning the production of E₂-isoprostanes in cardiovascular disorders, it is premature to conclude concerning the potential role of such compounds in cardiovascular pathogenesis.

One should keep in mind that Morrow et al. showed [44] that A₂-isothromboxanes are formed in vivo. Due to the inherent instability of the thromboxane A₂ ring, no data concerning the vascular effects of A₂-isothromb-
oxanes are available. However, regarding the potency of thromboxane \(A_2\)-induced contractions, studies using stable \(A_2\)-isothromboxanes analogues are awaited with interest.

To date, the metabolism of 15-F\(_{2\tau}\)-IsoP leads to two major metabolites in humans: 2,3-dinor-15-F\(_{2\tau}\)-IsoP and 2,3-dinor-5,6-dihydro-15-F\(_{2\tau}\)-IsoP [45,46]. The metabolite 2,3,4,5-tetranor-13,14-dihydro-15-keto-15-F\(_{2\tau}\)-IsoP [47] was identified as the major metabolite in rabbits but there is no evidence that such a metabolite is produced in humans. Although most prostanoid metabolites are biologically inactive, surprisingly, a recent report showed that 2,3-dinor-5,6-dihydro-15-F\(_{2\tau}\)-IsoP exhibits contraction that is comparable with that of 15-F\(_{2\tau}\)-IsoP in porcine brain microvessels [48]. By contrast, unlike 15-F\(_{2\tau}\)-IsoP, neither 2,3-dinor-5,6-dihydro-15-F\(_{2\tau}\)-IsoP nor 2,3-dinor-15-F\(_{2\tau}\)-IsoP had any constrictor or dilator effects on the rat thoracic aorta [49]. Such disparate observations need to be further investigated in different species and vascular beds.

Recently, isoprostane-like compounds derived from eicosapentaenoic and docosahexaenoic acids have been discovered in vivo [50–52]. Among the F\(_3\)-isoprostanes formed, 15-F\(_{3\phi}\)-IsoP possesses either no biological effect or might induce a weak relaxation in human airways. Preliminary data suggest that F\(_4\)-neuroprostanes possess no vascular effects.

**ISOPROSTANES AS A BIOMARKER OF LIPID PEROXIDATION IN VASCULAR DISEASES**

Isoprostanes have been measured in biological fluids such as urine, plasma, exhaled breath condensate, bronchoalveolar lavage fluid, bile, cerebrospinal, seminal and pericardial fluids. They are also detectable in normal tissues, including umbilical cords [53]. The main advantage of urinary measurements is that both 15-F\(_{2\tau}\)-IsoP and 5-F\(_{2\tau}\)-IsoP are not formed ex vivo by auto-oxidation in urine, unlike in plasma samples.

Cigarette smoking was one of the first conditions in which an increase in F\(_2\)-isoprostane levels was demonstrated [54]. This increase is reduced after 2 weeks of abstinence from smoking [14,54] and almost reaches the values of nonsmokers 4 weeks after quitting smoking [55]. Short-term cigarette smoking increases exhaled breath condensate F\(_2\)-isoprostane concentrations [56], but not plasma levels of F\(_2\)-isoprostane [54]. Restarting smoking after quitting and passive smoking are associated with an increase in plasma levels of F\(_2\)-isoprostane [57,58]. Interestingly, 15-F\(_{2\tau}\)-IsoP concentrations were approximately twice as high in umbilical cords from newborn babies of smoking mothers compared with those of nonsmoking mothers [53]. Together, these data provide evidence that cigarette smoking is associated with a chronic increased lipid peroxidation in vivo.

The measurement of isoprostanes in biological fluids has prompted clinical investigations on the pathophysiological role of lipid peroxidation in cardiovascular diseases (Table I). Among the biological fluids available, most studies were performed on urine because of the non-invasiveness of the procedure and the lack of artifactual generation. A strong link between lipid peroxidation and vascular diseases associated with ischemia–reperfusion, atherosclerosis and inflammation has been suggested by the elevated levels of lipid peroxidation observed in such diseases.

In addition to being a pathophysiological marker, the quantification of F\(_2\)-isoprostanes might represent a prognostic marker. Indeed, Schwedhelm et al. [59] showed in a case–control study that urinary 15-F\(_{2\tau}\)-IsoP level was a strong independent concentration-dependent risk marker of coronary heart disease. In addition, there is a relationship between plasma F\(_2\)-isoprostanes and early development of coronary artery calcifications [60]. There are currently no published clinical studies aimed at testing isoprostanes as a long-term prognostic marker, with strong endpoints such as mortality or morbidity, but cohort studies are on-going.

**ISOPROSTANES: EMERGING ROLE IN VASCULAR PHYSIOLOGY AND DISEASE?**

An important issue to resolve is whether the same effects observed in vitro are observed consistently in vivo at physiological concentrations and whether these effects contribute to pathological states in vivo. Basal plasma concentrations of 15-F\(_{2\tau}\)-IsoP have been found to range from approximately \(10^{-10}\) to \(5 \times 10^{-10}\) mol L\(^{-1}\) in plasma samples. These concentrations are unlikely to induce a systemic vasoactive effect considering the EC\(_{50}\) values of 15-F\(_{2\tau}\)-IsoP observed in vitro [21,61]. However, F\(_2\)-isoprostanes are released at the site of free radical injury and then diluted in the circulation and therefore local concentrations might be sufficiently high to induce regional vasoconstriction. The concentrations of 15-F\(_{2\tau}\)-IsoP and 5-F\(_{2\tau}\)-IsoP are increased markedly in the coronary sinus following coronary angioplasty [62]. However, 15-F\(_{2\tau}\)-IsoP concentrations are in the
Table I  F$_2$-isoprostane quantification in human cardiovascular diseases.

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<th>Disease</th>
<th>Isoprostanes quantified</th>
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<th>Method</th>
<th>Results in comparison with a control group</th>
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<td>Essential hypertension</td>
<td>15-F$_2$-IsoP</td>
<td>Urine</td>
<td>EIA and GC–MS</td>
<td>NSD</td>
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<td>15-F$_2$-IsoP</td>
<td>Urine</td>
<td>EIA</td>
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<td>Heart failure</td>
<td>15-F$_2$-IsoP</td>
<td>Urine, pericardial fluid</td>
<td>LC–MS–MS and EIA</td>
<td>Increased</td>
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<td>Atherosclerosis</td>
<td>15-F$_2$-IsoP and 5-F$_2$-IsoP</td>
<td>Atherosclerotic lesions from carotid endarterectomy</td>
<td>GC–MS and RIA</td>
<td>Increased</td>
<td>[93–95]</td>
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<td>Ruptured abdominal aortic aneurysm</td>
<td>15-F$_2$-IsoP</td>
<td>Plasma</td>
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<td>Stable coronary heart disease</td>
<td>15-F$_2$-IsoP</td>
<td>Urine</td>
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<td>Unstable angina</td>
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<td>Reperfusion following myocardial infarction and cardiopulmonary bypass</td>
<td>15-F$_2$-IsoP and 5-F$_2$-IsoP</td>
<td>Urine</td>
<td>GC–MS</td>
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<td>Coronary angioplasty</td>
<td>15-F$_2$-IsoP and 5-F$_2$-IsoP</td>
<td>Urine and coronary sinus</td>
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<td>Systemic sclerosis (scleroderma)</td>
<td>15-F$_2$-IsoP and F$_2$-isoprostane metabolites</td>
<td>Urine</td>
<td>GC–MS AND EIA</td>
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<td>Antiphospholipid antibodies syndrome</td>
<td>15-F$_2$-IsoP and 5-F$_2$-IsoP</td>
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<td>Pulmonary hypertension</td>
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<td>Acute ischemic stroke</td>
<td>15-F$_2$-IsoP</td>
<td>Urine</td>
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<td>Migraine</td>
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<td>15-F$_2$-IsoP and 5-F$_2$-IsoP</td>
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<td>Conflicting results among studies</td>
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EIA, enzyme immunoassay; GC–MS, gas chromatography–mass spectrometry; LC–MS–MS, liquid chromatography–tandem mass spectrometry; NSD, not significantly different; RIA, radioimmunoassay.
nanomolar range, and thus unlikely to contribute to epicardial coronary artery vasoconstriction [61,63].

No specific inhibition of 15-F_{2t}-IsoP or other isoprostanes vascular effects can currently be achieved. However, TP receptor antagonists but not aspirin are effective in atherosclerosis inhibition in apo E knock-out mice, showing that TP receptors blockade by S18886 is effective by a mechanism independent of platelet-derived thromboxane A₂ [64], whereas isoprostanes suppression with vitamin E retards atherogenesis in the same animal model [65]. Similarly, TP receptor antagonism by L670596, but not COX-2 inhibition prevented pulmonary hypertension and endothelin-1 upregulation in 60% O₂-mediated pulmonary hypertension in newborn rats [66]. In addition to these animal data, a recent study showed that in patients suffering from coronary artery disease, S18886, a TP receptor antagonist improved acetylcholine-induced and flow-mediated vasodilatation in patients treated with aspirin [67]. An hypothesis is that endogenous TP receptor activation induced by 15-F_{2t}-IsoP or other isoprostanes may be involved in the COX-independent effects of TP receptors antagonists [68]. However, because TP receptors share other endogenous ligands such as prostaglandin H₂ or HETEs, such data give strength to the hypothesis that isoprostanes are involved in the vascular physiology and pathogenesis, but does not enable a definitive conclusion. Given that many isomeric isoprostanes exist, one should not focus only on 15-F_{2t}-IsoP. Other 15-series F₂-isoprostanes are biologically active, as well as E₂-isoprostanes [22] and large field of investigations are still unexplored.

**ISOPROSTANES AS A PHARMACOLOGICAL TOOL FOR DRUG EVALUATION**

In the past decade, most attention has focused on the effect of an antioxidant therapeutic strategy, including the use of vitamin E, in cardiovascular and nephrological diseases, with mixed results. Measurement of F₂-isoprostanes currently represents a valuable pharmacological tool for the evaluation of antioxidant therapy, and should be used in the rational selection of antioxidant dosages. Vitamin E supplementation induced a reduction of urinary 15-F_{2t}-IsoP levels in patients with cystic fibrosis [69], type 2 diabetes [11] and homozygous homocystinuria [70], but not in patients with systemic sclerosis [71]. In addition, such a reduction was dose dependent in hypercholesterolemic patients [10]. The vitamin E-dependent reduction in the concentrations of F₂-isoprostane was observed in clinical conditions of enhanced oxidative stress. By contrast, supplementation with vitamin E had no effects on F₂-isoprostane levels in either moderate cigarette smokers [72], or healthy adults [73]. In the latter studies, the supplementation was associated with a significant dose-dependent increase in circulating concentrations of vitamin E. In addition, vitamin E supplementation in cigarette smokers on a high polyunsaturated fatty acid diet caused an increase in the plasma levels of F₂-isoprostane [74]. Together, these studies suggest that vitamin E supplementation has antioxidant effects in patient populations that are characterized by high rates of lipid peroxidation. Patrignani et al. [72] hypothesized that the basal rate of lipid peroxidation may be an important determinant of the response to vitamin E supplementation, and could explain the variable effects of vitamin E supplementation in large clinical trials. Several studies favor such a hypothesis. Oral treatment with raxofelast, a vitamin-E-like antioxidant, induced a significant reduction of plasma concentrations of 15-F_{2t}-IsoP in type 2 diabetes but had no effect in healthy subjects [75]. In addition, vitamin C supplementation reduced urinary 15-F₂-IsoP levels in patients with stroke [76], and chronic alcoholic liver disease but not in patients with hepatitis C cirrhosis, in whom endogenous vitamin C and E concentrations did not significantly differ compared with controls [77]. Furthermore, vitamin C did not decrease urinary F₂-isoprostane and metabolite levels in healthy young women [78], and in young subjects with a limited history of cigarette smoking [79]. Together, these data strengthen the need to incorporate the measurement of surrogate end-points such as F₂-isoprostanes in large-scale antioxidant clinical trials.

In addition to drug evaluation, F₂-isoprostane measurement could be used to test the antioxidant properties of the diet. F₂-isoprostane levels were decreased following supplementation with eicosapentaenoic acid or docosahexaenoic acid [80], fish meals in diabetic patients [81], olive oil [82], soy-containing isoflavone [83], gazpacho [84], and flavanol-rich cocoa [85].

**CONCLUSION**

Isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism. Some isoprostanes induce vasoconstriction, mitogenesis and monocyte adhesion. The quantification of F₂-isoprostanes as a pathophysiological biomarker provides a unique opportunity to investigate lipid...
peroxidation in vascular diseases. Their measurement provides an interesting biomarker for the rational dose selection of antioxidants in vascular diseases where oxidative stress might be involved. The data available supports but does not prove the hypothesis that isoprostanes are involved in vascular physiology and pathogenesis.

REFERENCES


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<td></td>
</tr>
<tr>
<td>Insert space between letters</td>
<td>linking 2 letters</td>
<td></td>
</tr>
<tr>
<td>Insert space between words</td>
<td>between letters affected</td>
<td></td>
</tr>
<tr>
<td>Reduce space between letters</td>
<td>between words affected</td>
<td></td>
</tr>
<tr>
<td>Reduce space between words</td>
<td>between letters affected</td>
<td></td>
</tr>
</tbody>
</table>