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Review

The isoprostanes—25 years later ☆

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ABSTRACT

Isoprostanes (IsoPs) are prostaglandin-like molecules generated independent of the cyclooxygenase (COX) by the free radical-induced peroxidation of arachidonic acid. The first isoprostane species discovered were isomeric to prostaglandin $F_{2\alpha}$ and were thus termed F_2-IsoPs. Since the initial discovery of the F_2-IsoPs, IsoPs with differing ring structures have been identified as well as IsoPs from different polyunsaturated fatty acids, including eicosapentaenoic acid and docosahexanenoic acid. The discovery of these molecules *in vivo* in humans has been a major contribution to the field of lipid oxidation and free radical research over the course of the past 25 years. These molecules have been determined to be both biomarkers and mediators of oxidative stress in numerous disease settings. This review focuses on recent developments in the field with an emphasis on clinical research. Special focus is given to the use of IsoPs as biomarkers in obesity, ischemia-reperfusion injury, the central nervous system, cancer, and genetic disorders. Additionally, attention is paid to diet and lifestyle factors that can affect endogenous levels of IsoPs. This article is part of a Special Issue entitled "Oxygenated metabolism of PUFA: analysis and biological relevance".

1. Introduction

January 2015 marks the 25th anniversary of the first report by Morrow and Roberts on the discovery of the isoprostanes (IsoPs), prostaglandin-like molecules generated *in vivo* in humans independent of the cyclooxygenase (COX) by the free radical-induced peroxidation of arachidonic acid [1]. Over the course of the past 25 years, more than 3800 articles have been published in the field of IsoP research by numerous investigators around the world. Numerous excellent reviews have been written describing the formation, chemical synthesis, and biological activities of the IsoPs as well as their potential use as biomarkers of disease. This review will provide a brief historical perspective on the discovery of the IsoPs with the primary focus on recent clinical research in the field.

2. Historical perspective

2.1. Discovery and formation of isoprostanes

Oxidative stress is characterized by the inability of the body's natural antioxidant defenses to detoxify and protect against pro-oxidant, and often pro-inflammatory, species. Production of reactive oxygen species (ROS), typically free radicals, is a hallmark of oxidative stress. Overproduction of ROS has been implicated in a variety of diseases yet much remains to be understood about mechanisms of oxidant injury in humans. Polyunsaturated fatty acids (PUFAs), such as arachidonic acid, are one target of free radical insult during oxidative stress. In the mid-1970s, it was shown that prostaglandin (PG)-like compounds could be formed *in vitro* by the non-enzymatic peroxidation of purified PUFA; however, this work had never been carried out beyond *in vitro* studies [2].

In the 1980s, a study showed that PGD_2 derived from COX is primarily metabolized *in vivo* in humans to 9α,11β-$PGF_{2\alpha}$ by the enzyme 11-ketoreductase [3]. In aqueous solutions, however, PGD_2 is an unstable compound that undergoes isomerization of the lower side chain and these isomers can also be reduced by 11-ketoreductase to yield isomers of 9α,11β-$PGF_{2\alpha}$ [4]. In studies undertaken to further characterize these compounds utilizing mass spectrometry, it was found that when plasma samples from normal volunteers were processed and analyzed immediately, a series of peaks were detected possessing characteristics of F-ring PGs. Interestingly, however, when plasma samples that had been stored at −20 °C for several months were reanalyzed, identical chromatographic peaks were detected but levels of putative PGF_2-like

Abbreviations: AD, Alzheimer's disease; AKI, Acute kidney injury; aSAH, Aneurysmal subarachnoid hemorrhage; BMI, Body mass index; CAPG, Coronary artery bypass grafting; COX, Cyclooxygenase; CPB, Cardiopulmonary bypass; CSF, Cerebral spinal fluid; DA, Ductus arteriosus; DHA, Docosahexaenoic acid; EP, Prostaglandin E_2 receptor; EPA, Eicosapentaenoic acid; GSH, Glutathione; I/R, Ischemic-reperfusion; IsoF, Isofuran; IsoP, Isoprostane; NP, Neuroprostane; PAF, Platelet activating factor; PG, Prostaglandin; PUFA, Polyunsaturated fatty acid; ROS, Reactive oxygen species; RTT, Rett syndrome; TBI, Traumatic brain injury; TP, Thromboxane receptor; WC, Waist circumference.

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compounds were up to 100-fold higher [1]. In addition, base-catalyzed hydrolysis of plasma lipids also yielded significant amounts of the PGF_2-like compounds. Antioxidants and reducing agents suppressed the formation of these compounds and CCl_4, a toxic agent known to generate free radicals in the liver through metabolism to the CCl_3•, dramatically increased the formation of these compounds in rats [1,6]. These experiments confirmed that the observed PGF_2-like compounds were generated *in vivo*, not by a COX-derived mechanism, but rather non-enzymatically by autoxidation of arachidonic acid. Morrow and Roberts termed this new class of compounds F_2-isoprostanes (IsoPs) because they were isomeric to $PGF_{2\alpha}$ [5,6].

A mechanism to explain the formation of the F_2-IsoPs from arachidonic acid is outlined in Fig. 1 [7]. Following abstraction of a bisallylic hydrogen atom and the addition of molecular oxygen to arachidonic acid to form a peroxyl radical, the peroxyl radical undergoes 5-*exo* cyclization and a second molecule of oxygen adds to the backbone of the compound to form PGG_2-like compounds. These unstable bicycloendoperoxide intermediates are then reduced to the F_2-IsoPs. Based on this mechanism of formation, four F_2-IsoP regioisomers, each of which is comprised of eight racemic diastereomers, a total of 64 compounds, are generated. The four regioisomer classes are named according to the carbon number on which the side chain hydroxyl group is attached (Fig. 1). This nomenclature system has been approved by the Eicosanoid Nomenclature Committee, which is sanctioned by JCBN of IUPAC [8]. An alternative nomenclature system for the IsoPs has been proposed by FitzGerald and colleagues in which the abbreviation iP is used for isoprostane, and the regioisomers are denoted as III-VI (Fig. 1) [9]. It is important to note that 5- and 15-series regioisomers are formed in significantly greater abundance than the 8- and 12-series regioisomers as 8- and 12-series regioisomers readily undergo further oxidation [10].

In addition to F_2-IsoPs, various classes of IsoPs that differ in regards to the functional groups on the prostane ring have been discovered; the structures of these different compounds are summarized in Fig. 2. The compounds are named based upon the structure of the functional

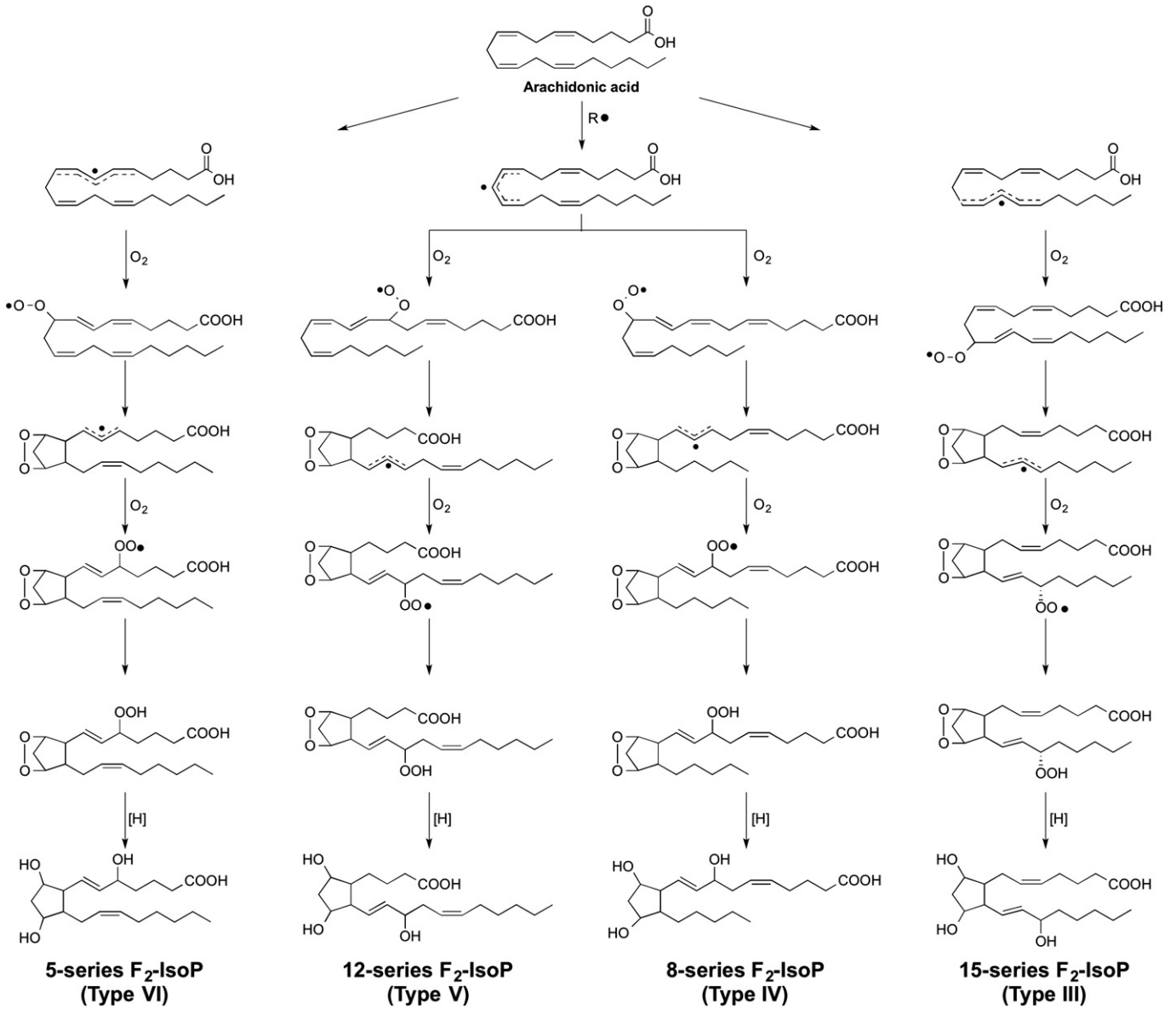

Fig. 1. Mechanism of formation of F_2-isoprostanes from the free radical-cataylzed peroxidation of arachidonic acid. Two primary nomenclature systems have been developed to classify isoprostanes [8,9]. In the nomenclature system used throughout this manuscript, IsoP is used as the abbreviation for isoprostane and the four regioisomer classes are named according to the carbon number on which the side chain hydroxyl group is attached, with the carboxyl carbon being ***1*** [8]. This nomenclature system has been approved by the Eicosanoid Nomenclature Committee, which is sanctioned by JCBN of IUPAC. The alternative nomenclature system uses the abbreviation iP for isoprostane and the regioisomers are denoted as III–VI based upon the number of carbons between the omega carbon and the first double bond [9].