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Forum Editorial

Introduction for Special Forum Issue on Isoprostanes and Related Compounds

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THIS ISSUE of *Antioxidants and Redox Signaling* is devoted to isoprostanes and related compounds. The isoprostanes are a series of prostaglandin-like molecules derived from the free radical-catalyzed peroxidation of arachidonic acid *in vivo* (11). Although they were originally discovered as products of arachidonate peroxidation *in vitro* several decades ago, there was little interest in them until it was shown in 1990 that they were formed in large amounts *in vivo* in humans (11, 12). Since that time, the field of isoprostane research has exploded and a current literature search reveals >1,000 citations related to these compounds.

The impact of the discovery of the isoprostanes has been important for two reasons. The first is that quantification of these compounds has become the gold standard for the assessment of oxidative stress status *in vivo* (4). As discussed by several authors in this Forum issue, the isoprostanes have been detected in increased amounts in a number of human diseases, and these observations allow for mechanistic insights into the pathophysiological sequelae of oxidant stress and also provide for potential therapeutic targets to ameliorate oxidative injury. A second important aspect of the isoprostanes is that a number of these compounds possess potent biological activity at nanomolar concentrations and, thus, may mediate certain consequences of oxidant stress (4). In particular, two isoprostanes that have been studied in detail, 15-F_{2t}-isoprostane (8-iso-prostaglandin $F_{2\alpha}$) and 15-E_{2t}-isoprostane (8-iso-prostaglandin E_2), exert significant biological effects by interaction with the G protein-coupled receptor, the thromboxane receptor, and possibly other uncharacterized receptors. As discussed herein, other isoprostanes exert a number of other biological activities.

The goals of this Forum issue are threefold. First, readers will be provided with an up-to-date date review regarding key aspects of isoprostane chemistry, mechanisms of isoprostane formation and metabolism, their biological activities, and their roles as markers and mediators of oxidant stress in human disease. Second, a number of the articles included herein contain data from unpublished or very recently published studies and thus provide readers with state-of-the-art information related to the isoprostanes. Finally, directions for future research that are likely to yield important insights into the biomedical aspects of isoprostanes and related compounds are provided.

This Forum issue contains 10 articles that have been authored by leaders in the field of isoprostane research. The first two provide important recent insights into mechanisms of fatty acid oxidation and factors that modulate the formation of isoprostanes and related compounds. In the first articles, Zemski Berry and Murphy examine the oxygenation of plasmalogen glycerophosphocholine containing docosahexaenoic acid (15). These studies are previously unpublished and explore a little studied area of lipid peroxidation involving plasmalogens. Plasmalogens are a unique class of choline and ethanolamine glycerophospholipids that have a vinyl ether moiety rather than an ester group at the *sn*-1 position of the glycerol backbone. Plasmalogens containing docosahexaenoic acid at the *sn*-2 position constitute a major portion of the phospholipids present in human brain and have been hypothesized to serve, in part, to protect against free radical attack. Docosahexaenoic acid-containing plasmalogens would be predicted to readily undergo peroxidation to yield a variety of oxidation products. Interestingly, though, the oxidation of these compounds has never been studied. In an elegant set of experiments, Zemski Berry and Murphy report that the oxidation of 1-*O*-hexadec-1′-enyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine yields large amounts of products resulting from oxidation at both the *sn*-1 and *sn*-2 positions (15). Significant oxidation at the *sn*-1 position is likely due to the reactivity of the vinyl ether moiety of the molecule. These important studies have, therefore, defined systematically, for the first time, the oxidation of docosahexaenoate-containing plasmalogen and will allow for a rational approach to exploring the biological significance of oxidized plasmalogens.

This work is complemented by the second article in this series, authored by Yin and Porter, who report exciting findings that they have made regarding the oxygenation of arachidonate (14). In particular, these authors have defined a unified mechanism for the oxygenation of arachidonic acid *in vitro*

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and *in vivo* and have shown that isoprostanes are a major product of this pathway. Further, they detail recent work that extends our understanding as to why even though isoprostanes are formed in abundance, certain regioisomers predominate over others. Utilizing state-of-the-art mass spectrometric approaches, they provide evidence that select isoprostane regioisomers undergo additional oxygenation to form a novel series of compounds termed dioxolane-isoprostanes that are generated at the expense of the isoprostanes. These findings have major import regarding the abundance of various isoprostanes produced *in vivo* and thus have significance with respect to which compounds should be studied for bioactivity.

In the third article in this Forum issue, Salomon describes his discovery and characterization of a group of highly reactive compounds termed levuglandins and isolevuglandins that are derived from rearrangement and cleavage of a carbon–carbon bond in the prostane ring of prostaglandin and isoprostane endoperoxides, respectively (13). These molecules are readily formed in aqueous solutions both *in vitro* and *in vivo*. They contain highly reactive γ-ketoaldehyde moieties that rapidly covalently adduct biological nucleophiles including, importantly, lysine residues on proteins. This extraordinary reactivity underlies many of the biological consequences of levuglandin and isolevuglandin generation. They have been shown to interfere with protein function and are among the most potent neurotoxins known. In his review, Salomon provides significant information on not only the chemistry and biochemistry of these compounds, but their relevance to human disease.

The isofurans are a recently discovered class of arachidonate peroxidation products that are related to, but distinct from, the isoprostanes. Importantly, their formation is favored at increasing oxygen tensions, especially above 21%. In their article, Fessel and Roberts review mechanisms related to the formation of the isofurans compared with that of the isoprostanes with an emphasis on the mechanistic basis for the favored formation of isofurans at elevated oxygen levels (5). The authors also discuss recent data showing that isofuran formation is selectively increased in human diseases associated with elevated oxygen tension, such as hyperoxic lung injury, and provide data to suggest that isofuran quantification, in certain settings, may augment isoprostanes to provide a more complete index of oxidant stress *in vivo*.

In the fifth article in this Forum issue, Milne and colleagues examine the biochemistry and biology of a group of isoprostanes that contain highly reactive unsaturated carbonyl moieties that are termed cyclopentenone isoprostanes (8). These compounds have structures analogous to cyclooxygenase-derived prostaglandin A_2 and prostaglandin J_2. Importantly, however, unlike cyclopentenone prostaglandins, the cycloopentenone isoprostanes have been shown to be formed in large amounts *in vivo*, providing a rationale to explore their bioactivity. It has recently been shown that cyclopentenone isoprostanes readily undergo Michael addition with various thiols such as glutathione and, indeed, these compounds are primarily metabolized in cells to glutathione adducts (7). This pathway of metabolism also likely occurs to a significant extent *in vivo*. In addition, evidence is presented for the first time that cyclopentenone isoprostanes possess potent bioactivity and modulate inflammatory mediator release in macrophages. A potential role for these compounds in the pathophysiology of certain human diseases is also discussed.

Basu and Helmersson provide important information for readers in their article on factors that regulate isoprostane formation *in vivo* in humans (2). This work is an important resource that has not heretofore been compiled. Of note, isoprostane levels are readily influenced by factors, including gender, ethnicity, and various lifestyle modifiers. Such factors clearly should be considered when assessing the impact of disease processes and various interventions on endogenous oxidant stress in humans.

The last four articles in this Forum issue examine the role that isoprostanes play in the pathophysiological consequences of oxidant stress in various tissues and human disease states. In their article, Badr and Abi-Antoun explore the biological activities of isoprostanes in the kidney (1). The potent effect of one isoprostane, 15-F_{2t}-isoprostane, as a vasoconstrictor was first documented in the renal vasculature (11). In addition, the molecular signaling mechanisms related to the bioactivity of 15-F_{2t}-isoprostane were first discerned in this tissue when it was shown that 15-F_{2t}-isoprostane exerted its vasoconstricting properties by interacting with the thromboxane receptor. Badr and Abi-Antoun summarize, in a concise manner, the current knowledge regarding these and other effects of various isoprostanes in the kidney, with a particular focus on intracellular signaling mechanisms. In addition, they discuss a still unanswered question as to whether specific "isoprostane" receptors are present in various tissues.

Janssen and colleagues subsequently examine the pulmonary biology of isoprostanes (6). These compounds exert a variety of receptor-mediated and receptor-independent effects on a wide variety of cell types in the lung, including smooth muscle, neurons, epithelium, and lymphatics. The detailed signaling mechanisms responsible for a number of these activities have been elegantly elucidated by Janssen over the years, and this chapter in which they are described will provide a wealth of information for pulmonary physiologists.

Isoprostanes have been extremely useful in examining the role of oxidant injury in association with risk factors for atherosclerosis. Chief among these risk factors is diabetes mellitus. Davi and colleagues, in their chapter, explore the role of oxidant stress in the pathogenesis, at a molecular level, of both type 1 and type 2 diabetes mellitus and discuss recent studies examining the effects of hyperglycemia and metabolic control on isoprostane formation (3). The consequences of isoprostanes on platelet activation are also discussed, as is the role of antioxidant therapy in the prevention or amelioration of diabetic vascular complications.

The final chapter in this Forum issue by Montine and colleagues deals with the role of isoprostane formation in neurodegenerative disorders (9). Increased free radical-mediated injury to brain is proposed to be an integral component of several neurodegenerative diseases, including Alzheimer's disease. Montine has previously shown that F_2-isoprostane concentrations are selectively increased in diseased regions of brains from patients who died from advanced Alzheimer's disease, where pathologic changes include amyloid β amyloidogenesis, neurofibrillary tangle formation, and extensive neuron death. Interestingly, cerebral F_2-isoprostanes are not

reproducibly elevated in aged mouse models of cerebral amyloid β amyloidogenesis only. There is broad agreement that increased cerebrospinal fluid levels of F_2-isoprostanes also are present in patients with early Alzheimer's disease. Demonstrated applications of quantifying cerebrospinal fluid F_2-isoprostanes have improved laboratory diagnostic accuracy of Alzheimer's disease and objective assessment of antioxidant therapeutics. As discussed in this article, this work indicates that brain lipid peroxidation is a potential therapeutic target early in the course of Alzheimer's disease, and that cerebrospinal fluid F_2-isoprostanes may aid in the assessment of antioxidant experimental therapeutics and laboratory diagnosis of Alzheimer's disease.

An important goal of the articles presented in this Forum issue is to provide a platform for future studies related to the isoprostanes. Although discovery of these compounds has greatly increased our knowledge of the pathophysiology of oxidative injury and the role oxidant stress plays in human diseases, there are many areas of isoprostane research that have not been investigated. In particular, a better understanding of the role of these compounds as mediators of oxidative injury is needed. Although the bioactivity of certain isoprostanes has been characterized, how this activity translates into these compounds being causative mediators of tissue injury is not clear. In addition, the bioactivities of only a few isoprostanes have been determined, in large part because only a few have been chemically synthesized (4). Synthesis of additional compounds and examination of their bioactivity will likely also advance our understanding of the roles of isoprostanes as mediators of oxidant injury. In addition, a clearer elucidation of factors involved in the formation and metabolic disposition of the isoprostanes will also advance our knowledge of the biology of these compounds.

A second need in the area of isoprostane research is the further development of facile and robust methods to quantify these compounds. Although not discussed in this Forum issue in detail, accurate assays to measure these compounds, and therefore assess the role of oxidative injury in disease processes, involve complex and expensive mass spectrometric methodologies. Immunoassay approaches utilizing polyclonal antibodies have been developed although they are not precise or robust. The development of reproducible, sensitive methods that can be performed using a fairly simple method would therefore greatly facilitate research in this area.

A third area for future investigation related to the isoprostanes is the development of therapeutic interventions to decrease their formation. Clearly, oxidant stress and isoprostane formation are increased in a number of human diseases, and this has been linked to certain pathophysiological sequelae (4). With the development of accurate methods to quantify oxidant stress *in vivo*, the next milestone to achieve will be to effectively suppress it and determine the effect of suppression on human pathophysiology. Putative antioxidants such as vitamin E and vitamin C are, at best, weak inhibitors of isoprostane formation *in vivo* (10), and so the development of better, more effective therapeutic agents is clearly needed.

In summary, the focus of this Forum issue is the isoprostanes and related compounds. Their discovery *in vivo* a little over a decade ago has spawned an area of intense and active research related to the role of oxidative injury in human physiology and pathophysiology. The articles contained in this issue are designed to provide the reader with cutting-edge information about this important group of oxidized lipids.

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