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Mini Review Article

Isoprostanes and Related Compounds: Update 2006

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ABSTRACT

Last year *Antioxidants and Redox Signaling* devoted a special Forum issue (Volume 7, 2005) to reviewing the current literature on isoprostanes (IsoPs) and related compounds. A wide variety of topics provided readers of that issue with important information on the chemistry and biology of these molecules. During the past year, more than 200 articles have been published in the field of IsoP research, and noteworthy advances in the area have been made. This editorial highlights selected aspects of these advances as they relate to the Forum issue. The main focus of this discussion is on the role of the IsoPs as biomarkers and mediators of oxidant stress in human disease, their mechanism of formation and metabolism, and their biologic activities. *Antioxid. Redox Signal.* 8, 1379–1384.

LAST YEAR, *Antioxidants and Redox Signaling* devoted a special Forum issue (Volume 7, 2005) to reviewing the current literature on isoprostanes (IsoPs) and related compounds (2–5, 7, 10, 11, 16, 18, 19, 21, 26). In that issue, a wide variety of topics provided readers with important up-to-date information on the chemistry and biology of these molecules. During the past year, more than 200 articles have been published in the field of IsoP research, and significant advances in the area have been made. This editorial highlights selected aspects of these advances as they relate to the Forum issue. The primary focus of this discussion is on the role of the IsoPs as markers and mediators of oxidant stress in human disease, their mechanism of formation and metabolism, and their biologic activities.

IMPORTANCE OF THE ISOPROSTANES AS MARKERS OF OXIDANT STRESS: AN UPDATE

As is evidenced by the wide variety of topics discussed by many authors in last year's Forum issue, the impact of the discovery of the IsoPs has been important for many reasons. One of the most important outcomes is that the quantification of these compounds has become the "gold standard" for the assessment of oxidative-stress status *in vivo*. In the past year, this supposition was confirmed in the second report of the Biomarkers of Oxidative Stress Study (BOSS) (12). This multilaboratory validation study, organized and sponsored by the National Institute of Environmental Health Sciences (NIEHS), sought to determine which of the common biomarkers used for noninvasive measurement of oxidative-stress status *in vivo* were most specific, sensitive, and selective. The model of oxidative stress used in this study was the CCl_4 poisoning of rodents; biomarkers were measured at multiple doses and at multiple times after administration of CCl_4. In this study, plasma and urinary 8-isoprostane-prostaglandin F ($PGF_{2\alpha}$) (8-iso- $PGF_{2\alpha}$ or 15-F_2-IsoPs) were measured by multiple laboratories and methods and showed well-defined time- and dose-dependent increases after CCl_4 treatment. These molecules were found to be the most valid and sensitive measures of oxidative-stress status *in vivo*.

UPDATE ON FACTORS REGULATING ISOPROSTANE FORMATION

As the IsoPs are such important biomarkers of oxidant stress, an entire body of literature details factors that regulate

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IsoP formation *in vivo*. In their review last year, Basu and Helmersson (3) provided readers with the first compilation summarizing many of these controlling factors, including physiologic factors, such as gender and ethnicity; exogenous factors, including diet, exercise, smoking, and medications; and disease. During the past year, several studies have brought new insights into exogenous factors and diseases that can regulate *in vivo* formation of IsoPs. Selected aspects of these studies are discussed herein.

The first study of interest was conducted by last year's authors, Helmersson, Basu, and colleagues (9). In this report, the effect of selenium (Se) on *in vivo* indicators of oxidative stress and inflammation was investigated in a 27-year follow-up study of Swedish men. Specifically, serum Se was measured in a population of Swedish men at age 50 years, and the status of oxidative stress and inflammation was evaluated in a repeated investigation 27 years later. The authors found an inverse association between levels of Se and urinary 8-iso-$PGF_{2\alpha}$, independent of confounding factors including body mass index (BMI), diabetes, hyperlipidemia, hypertension, smoking, α-tocopherol, and β-carotene. These results indicate that higher levels of Se are related to less oxidative stress, which is consistent with previous findings that showed that low concentrations of Se might be a risk factor for accelerated atherogenesis, development of cardiovascular diseases (CVDs), and mortality in some populations. The fact that these authors did find an association between serum Se and urinary F_2-IsoPs in such a long follow-up study suggests that these results have clinical relevance and provide insight into the proposed antiatherogenic properties of Se.

In the next two studies described herein, the influence of exogenous factors on *in vivo* IsoP formation are discussed. One widely studied exogenous factor that is associated with significantly higher levels of 8-iso-$PGF_{2\alpha}$ in plasma, urine, breath condensate, and in some tissues is cigarette smoking. Although the direct effects of smoking are well documented, data on isoprostanes and passive smoking are lacking. Several studies have shown that passive smoking is associated with an increased risk of coronary artery disease. As reported by Sinzinger and colleagues in 2003 and as mentioned by Basu and Helmersson (3) in their review, children of smoking parents have increased formation of F_2-isoprostanes (23). Recently, Sinzinger's laboratory (1) reported a study examining the effect of passive smoking on F_2-IsoP levels, as measured by quantifying plasma 8-epi-$PGF_{2\alpha}$, in adults. In this study, both smokers and nonsmokers were exposed to the smoke of 30 cigarettes for 60 min once daily over a period of 12 days. Immediately after this exposure to passive cigarette smoke, levels of the IsoP increased by 30% in nonsmokers and 15% in smokers. Levels returned to baseline 6 h after exposure. However, after repeated exposure during the 12-day period, baseline levels of the IsoP steadily increased, as did the values measured 6 h after exposure, suggesting that repeated but not single exposure to passive smoking introduces a sustained oxidant stress *in vivo*.

A second exogenous factor that has been highly studied is the effect of antioxidant consumption on endogenous levels of oxidized lipids. As fruits and vegetables are rich in antioxidants, it is thought that ingestion of a diet rich in these foods could inhibit *in vivo* IsoP formation. However, as reported by Basu and Helmersson (3), a search of the literature in this area yields conflicting results. Recently, in light of these varying results, Thompson and colleagues (24) reported a clinical intervention study that tested the hypothesis that increased vegetable and fruit (VF) consumption decreases oxidative stress, as measured by urinary excretion of 8-iso-$PGF_{2\alpha}$. In this study, for the initial phase, participants were given a balanced diet with two to four servings of VF; after 2 weeks, participants were assigned to either a low-VF diet (three to four servings of VF) or a high-VF diet (eight to 10 servings) for 4 weeks, and all participants consumed the high-VF diet for the last 2 weeks. It was found that the initial diet served in the first 2 weeks of the study caused a 33% decrease in IsoPs. The high-VF diet subsequently caused another 14% decrease in IsoPs after 2 weeks, whereas no further decrease was observed with the low-VF diet. However, when participants receiving the low-VF diet switched to the high-VF diet at the end of the study, a statistically significant decrease in IsoPs was observed. The greatest reductions in 8-iso-$PGF_{2\alpha}$ were observed in the subjects with the highest quartile of baseline concentrations. These findings are of significance, as this is the first report of an intervention study that shows that high intake of fruits and vegetables produces an antioxidant effect *in vivo*.

UPDATE ON ISOPROSTANE FORMATION IN HUMAN DISEASES

In addition to these studies that further explored the relation between exogenous factors and *in vivo* IsoP levels, in the past year, several studies examined the formation of IsoPs in human disease. Two studies of interest report increased levels of F_2-IsoPs in human disorders that heretofore had been thoroughly explored—chronic fatigue syndrome and autism (13, 17). In the first study, Kennedy and co-workers (13) reported that levels of plasma 8-iso-$PGF_{2\alpha}$ are increased in normotensive, nonobese patients with chronic fatigue syndrome as compared with matched controls (13). In addition, these authors showed that IsoP levels are positively correlated with symptoms of the disease, including joint pain and postexertional malaise. This study examining the relation between chronic fatigue syndrome and plasma F_2-IsoP levels is the first of its kind, but the role of IsoPs in the pathogenesis of the disease is not known. In the second study of interest, Ming and colleagues (17) reported that levels of urinary 8-iso-$PGF_{2\alpha}$ were approximately 6 times higher in children with autism as compared with age-matched controls. Furthermore, the group distribution of these data was examined, and they reported that a subgroup of children with autism have extremely high levels of urinary 8-iso-$PGF_{2\alpha}$ ($\leq$46-fold higher than controls). The authors did not find any confounding factors associated with this increase in IsoPs in this cohort of children; but they as yet have not been able to define any phenotypic, genotypic, or biologic traits associated with these patients. Together these studies confirm the utility of these molecules as biomarkers but show that still much research is to be done examining the relation between oxidative stress, the IsoPs and other bioactive lipids, and the pathogenesis of human disease.

In addition to these reports, which highlight new relations between *in vivo* IsoP formation and human disease, several reports concerned the formation of IsoPs in the context of some of the diseases highlighted in the Forum issue. Specifically, diseases of the lung and central nervous system are discussed.

In last year's review by Janssen and colleagues (11), the extensive pulmonary biology of IsoPs was explored. As discussed in that article, the IsoPs 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. Because of this array of biologic activities, it was suggested that these effects could culminate in the manifestation of asthma. At the time of last year's review, it was known that 8-iso-$PGF_{2\alpha}$ accumulates in the lungs in asthma and is increased in the exhaled breath condensate (EBC) of adults with the disease. Recently, Shahid and co-workers (22) reported that 8-iso-$PGF_{2\alpha}$, as measured by immunoassay, is increased in the EBC of children with asthma, including those receiving inhaled steroid therapy. Although this finding is not unexpected, based on the adult studies, and the assay would be more robust if IsoP-levels were measured by mass spectrometry rather than by immunoassay, it is of interest because the authors suggest the use of this measurement as a noninvasive procedure to assess airway inflammation in asthmatic children, as traditional invasive procedures are not possible in children. In addition, as they were able to show that treatment with inhaled steroids, the mainstay of treatment in childhood asthma, does not decrease levels of 8-iso-$PGF_{2\alpha}$ in EBC. The authors suggest that other treatments such as antioxidants should be explored.

In the last article of the Forum issue, Montine and colleagues (18) dealt with the role of IsoP formation in neurodegenerative disorders. Increased free radical–mediated injury to brain is proposed to be an integral component of several neurodegenerative diseases, including Alzheimer disease (AD). As was discussed in last year's article, quantifying cerebrospinal fluid F_2-IsoPs has improved laboratory diagnostic accuracy of AD and objective assessment of antioxidant therapeutics. In the past year, several articles have expanded the use of quantifying CSF IsoPs as a diagnostic tool as well as our knowledge of the pathogenesis of AD. In the first article, Grossman and co-workers (8) assessed CSF levels of a number of biomarkers of AD. The biomarkers quantified included F_2-IsoPs; tau, a microtubule-associated protein whose metabolism, when it is disrupted, is associated with a number of neurodegenerative conditions; and $A\beta_{1-42}$, a marker of the accumulation of abnormally cleaved amyloid Aβ. Their studies found that these biomarkers, when quantified together, yield a CSF biomarker profile that distinguishes frontotemporal dementia (FTD) disorders from AD. Specifically, as compared with patients with AD, patients with an FTD disorder have lower levels of tau, lower levels of F_2-IsoPs, and higher levels of $A\beta_{1-42}$. Furthermore, by using the measured levels of these three biomarkers nearly 90% of the studied patients were classified in a manner that corresponded to their clinical or autopsy diagnosis.

In addition to this study, two studies were published within 1 month of each other and more closely looked at the role of lipid peroxidation in amnestic mild cognitive impairment (MCI), the earliest detectable clinical phase of AD. In the field of neurodegenerative diseases as whole, a recent emphasis has been on early detection because of the hope that early treatment might slow disease progression. In the first study, de Leon and colleagues (6) performed the first reported longitudinal study of normal and MCI subjects examining CSF biomarkers, including F_2-IsoPs, hyperphosphorylated tau, and $A\beta_{1-42}$; memory; and hippocampal volume. The results of the CSF biomarker measurements in MCI patients were consistent with results previously observed for AD patients including the study by Grossman *et al*. described above; these patients have elevated levels of hyperphosphorylated tau, elevated F_2-IsoPs, and decreased Ab_{1-42} levels as compared to controls. In addition, MCI patients had decreased memory and hippocampal volumes, as is consistent with the pathogenesis of AD. Not surprisingly, the authors found that measuring the CSF biomarkers significantly improved diagnosis as compared with memory measures alone. Interestingly, the authors also showed F_2-IsoPs were the only measure that demonstrated significant longitudinal elevations in MCI over the 2-year study interval and that F_2-IsoPs correlated well with hippocampal volume. This finding is significant because it is the first longitudinal biomarker of MCI and suggests that lipid peroxidation could be playing a role in the pathologic progression of MCI and transition to AD. Furthermore, these findings are of interest in relation to a study by Markesberry and colleagues (14) published a few weeks later. In this study, levels of F_2-IsoPs as well as F_4-neuroprostanes (NPs, see discussion of formation later) were measured in different regions of the brains from longitudinally followed, autopsied control, MCI, and late-stage AD patients. These studies showed that both F_2-IsoPs and F_4-NPs were increased in all regions of the brain from both MCI and late-stage AD patients as compared with controls. However, the only statistically significant increase noticed in late-stage AD patients compared with MCI patients was in levels of F_4-NPs in the hippocampus. Although no longitudinal increase in IsoP levels was noted, as was observed in the CSF of MCI patients, the data reported in both the de Leon and Markesberry studies are highly significant because it demonstrates that lipid peroxidation is observed in the brain in even the earliest stages of AD. These observations suggest that oxidative damage plays a role in the pathogenesis of the disease and is not a late effect of the neurodegenerative process. This data also opens therapeutic possibilities for the use of antioxidants in patients in the early phase of the disease and for those who are at high risk for AD.

Update on mechanisms of isoprostane formation

Despite the vast amount of research that has been done examining the formation of IsoPs *in vivo* in the context of human conditions and diseases, still much work is required to elucidate fully the chemical mechanisms of lipid oxidation and *in vivo* IsoP formation and subsequent reaction/metabolism. However, several articles have made progress in these areas in the past year. In the Forum issue, Yin and Porter (26) defined a unified mechanism for the oxidation of arachidonic acid *in vitro* and *in vivo* and showed that IsoPs are a major product of this pathway (26). Furthermore, they detailed work

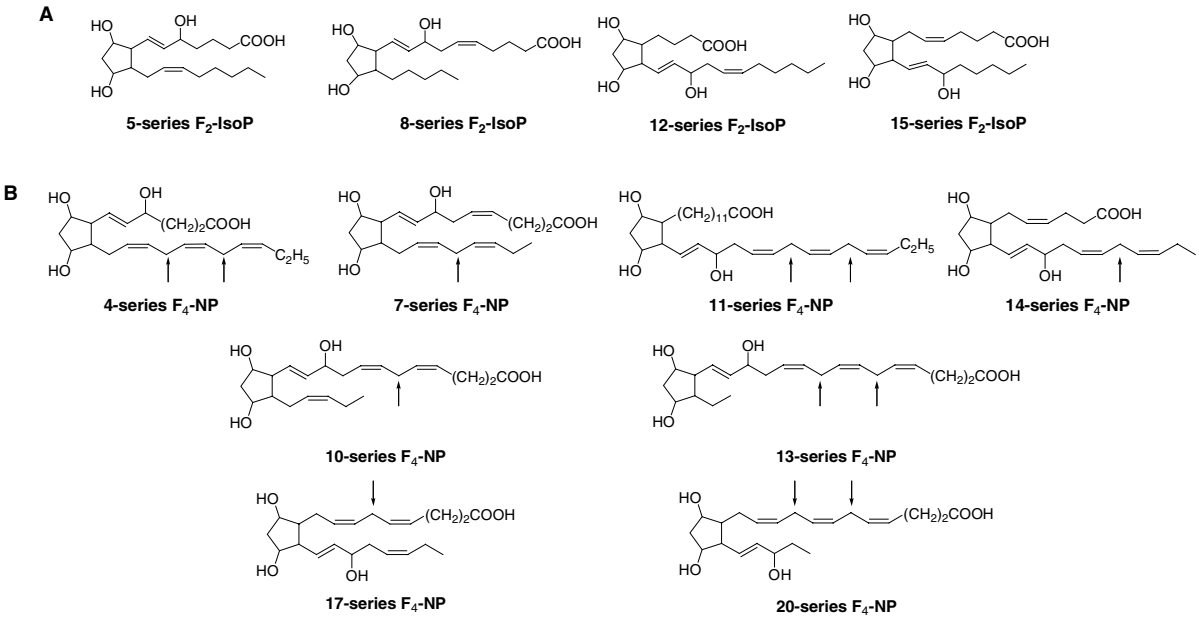

FIG. 1. (A) Structures of the four series of F_2-isoprostane regioisomers generated from the free radical oxidation of arachidonic acid. (B) Structures of the eight series of F_4-neuroprostane regioisomers generated from the free radical oxidation of docosahexaenoic acid. *Arrows*, Presence of abstractable bisallylic hydrogens.

that extended our understanding as to why 5- and 15-series IsoP regioisomers (Fig. 1A) are formed predominantly over others. By using mass spectrometric approaches, they provided evidence that 8- and 12-series IsoP regioisomers undergo additional oxidation to form a novel series of compounds termed dioxolane-isoprostanes, which are generated at the expense of the isoprostanes. Recently, these authors applied their understanding of the free-radical–based mechanism of arachidonic acid oxidation to characterizing the oxidation of docosahexaenoic acid (DHA), a polyunsaturated fatty acid (PUFA) that is highly enriched in the brain and is one of the major components of fish oil (25). Previously, it was reported that isoprostane-like compounds, termed neuroprostanes (NPs), are generated from the free radical oxidation of DHA and, as discussed earlier, levels of F-ring NPs (F_4-NPs) are elevated in the CSF from patients with Alzheimer disease. In this recent report, Yin and colleagues (25) demonstrated that, like the IsoPs generated from arachidonic acid, certain NP regioisomers predominate over others. As predicted, 4-series and 20-series NPs regioisomers (Fig. 1B) are preferentially generated from the autoxidation of DHA both *in vitro* and *in vivo*. They proposed that like the 5- and 15-series IsoPs, these compounds predominate because the precursors that lead to formation of the other regioisomers (7-, 10-, 11-, 13-, 14-, and 17-series NPs, Fig. 1B) can be further oxidized to dioxolane-IsoP–like compounds (dioxolane-NPs). However, whereas 5- and 15-series IsoPs are formed in 10 times greater abundance than 8- and 12-series IsoPs, the 4- and 20-series NPs are formed in only one-to threefold greater abundance than the other regioisomers. The authors reason that this is because the oxidation of DHA is much more complicated than that of arachidonic acid; thus dioxolane-NP formation contributes less to the NP regioisomer distribution than does IsoP regioisomer distribution. For example, because of the presence of bis-allylic positions on their backbone, the parent NPs can be peroxidized to generate oxidized species other than dioxolane-NPs. Several different classes of highly oxidized compounds could be generated as the 4-, 11-, 13-, and 20-series parent NPs have two bis-allylic positions, whereas the 7-, 10-, 14-, and 17-series NPs have one such position (arrows indicate these positions in Fig. 1B). Nevertheless, this discovery of regioselectivity in the formation of NPs represents the first mechanistic study on the autoxidation of DHA. These results will allow studies on the biologic activities of NPs to focus on the more abundantly generated compounds to determine their role in modulating the pathophysiologic consequences of DHA oxidation and oxidant stress.

Update on the biology of the isoprostanes

In addition to advances in understanding the chemical mechanisms controlling the regioselectivity of isoprostane and neuroprostane formation *in vitro* and *in vivo*, in the past year, key advances in understanding the metabolism and bio-

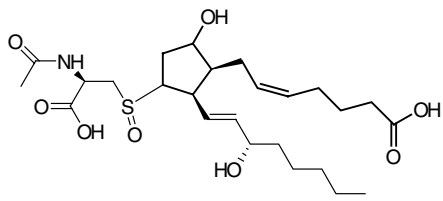

FIG. 2. Structure of the major urinary metabolite of 15-A_{2t}-IsoP in the rat.

logical activity of a group of highly reactive IsoPs termed the cyclopentenone IsoPs. In last year's forum issue, Milne and colleagues (16) reviewed the literature regarding the reactivity and metabolism of these compounds. Since last year's report, the major urinary metabolite of one cyclopentenone IsoP known to be formed abundantly *in vivo*, 15-A_{2t}-IsoP, has been identified in the rat (15). This metabolite, the structure of which is shown in Fig. 2, is a modified *N*-acetyl cysteine conjugate in which the cysteinyl sulfur is oxidized to the sulfoxide and the ketone on the prostane ring is reduced to the hydroxyl. The identification of this metabolite is significant because heretofore quantification of the cyclopentenone IsoPs *in vivo* as the free acids was not possible, as these molecules are not detectable circulating in plasma or excreted in urine, presumably because of their reactivity. To this end, the authors used this major urinary metabolite of 15-A_{2t}-IsoP as a biomarker to assess cyclopentenone IsoP formation *in vivo* in rats. They were able to quantify for the first time cyclopentenone IsoP metabolite formation in rats treated with carbon tetrachloride (CCl_4), a potent inducer of *in vivo* oxidant stress, as well as in control animals.

In addition to reviewing the literature on the cyclopentenone IsoPs, Milne and colleagues reported for the first time in the forum issue that cyclopentenone IsoPs possess potent bioactivity and modulate inflammatory mediator release in macrophages. Since that time, this work has been expanded into a full manuscript (20). Therein, Musiek *et al*. showed that both 15-A_2- and 15-J_2-IsoPs potently inhibited the lipopolysaccharide-stimulated inflammatory response in RAW264.7 and primary murine macrophages. The mechanism by which the cyclopentenone IsoPs exerted this biologic activity was explored, and they found that these molecules inhibit IkBa degradation and subsequent nuclear factor (NF)-κB nuclear translocation and transcriptional activity. Expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were also inhibited by cyclopentenone IsoPs, as was production of nitrite and prostaglandin D_2. Additionally, the authors showed that the 15-J_2-IsoPs induced RAW cell apoptosis and were potent agonists for the peroxisome proliferators-activated receptor γ (PPARγ), but these effects were not observed for the 15-A_2-IsoPs. However, the biologic activities of both molecules were found to be PPARγ independent. These findings have important implications for defining the role of oxidant stress in the inflammatory response, as the cyclopentenone IsoPs, which could be generated during inflammation-induced oxidant stress, may serve as negative-feedback regulators to prevent excessive tissue damage during inflammation. Furthermore, as NF-κB, PPARγ, COX-2, and iNOS play important roles in the pathogenesis of many disease states, the biologic action of cyclopentenone IsoPs has broad implications for understanding of the role of oxidative stress in human pathophysiology.

SUMMARY

In conclusion, the discovery of the IsoPs *in vivo* a little over 15 years ago has spawned an area of intense and active research related to the role of oxidative injury in human physiology and pathophysiology. Articles contained in last year's Forum issue compiled cutting-edge information about this important group of oxidized lipids. Herein, we provide the reader with an update to last year's topics of discussion by bringing together several exciting aspects of IsoP research reported in the past year. As described, significant advances continue to be made in many different aspects of IsoP research, including advances in the understanding of their *in vivo* formation and metabolism, involvement in disease pathology, chemical mechanisms of formation, and biologic activity.

ABBREVIATIONS

AD, Alzheimer disease; BMI, body mass index; BOSS, Biomarkers of Oxidative Stress Study; COX-2, cyclooxygenase-2; CSF, cerebrospinal fluid; CVD, cardiovascular disease; DHA, docosahexaenoic acid; FTD, frontotemporal dementia; iNOS, inducible nitric oxide synthase; IsoP(s), isoprostane(s); 8-iso-$PGF_{2\alpha}$, 8-isoprostane-$PGF_{2\alpha}$; MCI, mild cognitive impairment; NIEHS, National Institute of Environmental Health Sciences; NP(s), neuroprostane(s); PPARγ, peroxisome proliferators-activated receptor γ; PUFA, polyunsaturated fatty acid; Se, selenium;VF, vegetables and fruit.

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