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F_2-Isoprostanes as markers of oxidative stress *in vivo*: An overview

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Abstract
The isoprostanes are a unique series of prostaglandin-like compounds formed *in vivo* via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid. This article summarizes selected aspects regarding current knowledge of these compounds and their value as markers of oxidative injury. Novel aspects related to the biochemistry of isoprostane formation are discussed and methods by which these compounds can be analysed and quantified are summarized. A considerable portion of this article examines the utility of F_2-isoprostanes as markers of oxidant injury *in vivo*. Numerous studies carried out over the past decade have shown that these compounds are extremely accurate measures of lipid peroxidation and have illuminated the role of oxidant injury in a number of human diseases including atherosclerosis, Alzheimer's disease and pulmonary disorders.

Keywords: *Lipid peroxidation, eicosanoid, isoprostane, lipid, oxidant stress, oxidative stress*

Abbreviations: *TBARS, thiobarbituric acid reacting substances, IsoP, isoprostane, PG, prostaglandin, GC, gas chromatography, NICI, negative ion chemical ionization, MS, mass spectrometry, AD, Alzheimer's disease, CSF, cerebrospinal fluid*

Introduction

The oxidation of cellular lipids, typically referred to as lipid peroxidation, is a central feature of oxidant stress, a phenomenon that has been increasingly implicated as playing a causative role in the pathophysiology of a number of human diseases. Lipid peroxidation, which is typically initiated by highly reactive free radical species, can be assessed by many methods including the measurement of either primary or secondary peroxidation end products. Primary end products of lipid peroxidation include conjugated dienes and lipid hydroperoxides, while secondary end products include thiobarbituric reactive substances (TBARS), gaseous alkanes and a group of prostaglandin (PG) F_2-like products termed F_2-isoprostanes (F_2-IsoPs) (Halliwell and Grootveld 1987, Morrow et al. 1990a). Quantification of these various compounds has proven highly useful for the study of free radical-mediated lipid peroxidation in *in vitro* model systems. However, the F_2-IsoPs appear to be a

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significantly more accurate marker of oxidative stress *in vivo* in humans and animals than other compounds (Liu et al. 1999, Morrow and Roberts 1999, Fam and Morrow 2003). Herein, considerations regarding the use of F_2-IsoPs as specific, reliable and non-invasive biological markers of *in vivo* oxidative stress will be discussed.

Mechanism of formation of isoprostanes

IsoPs are prostaglandin (PG)-like compounds formed from the peroxidation of arachidonic acid, a ubiquitous polyunsaturated fatty acid (Morrow et al. 1990a, Morrow and Roberts 1999). Unlike PGs, which are formed via the action of the cyclooxygenase enzymes, F_2-IsoPs are formed non-enzymatically as a result of the free radical-mediated peroxidation of arachidonic acid. Figure 1 outlines the mechanism by which IsoPs are generated. Following abstraction of a bisallylic hydrogen atom and the addition of a molecule of 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 are generated (see Figure 1) (Morrow et al. 1990a, b, Morrow and Roberts 1999). Compounds are denoted as 5-, 12-, 8- or 15-series regioisomers depending on the carbon atom to which the side chain hydroxyl is attached (Taber et al. 1997).

IsoPs that contain F-type prostane rings are isomeric to $PGF_{2\alpha}$ and are, thus, referred to as F_2-IsoPs. It should be noted that IsoPs containing alternative ring

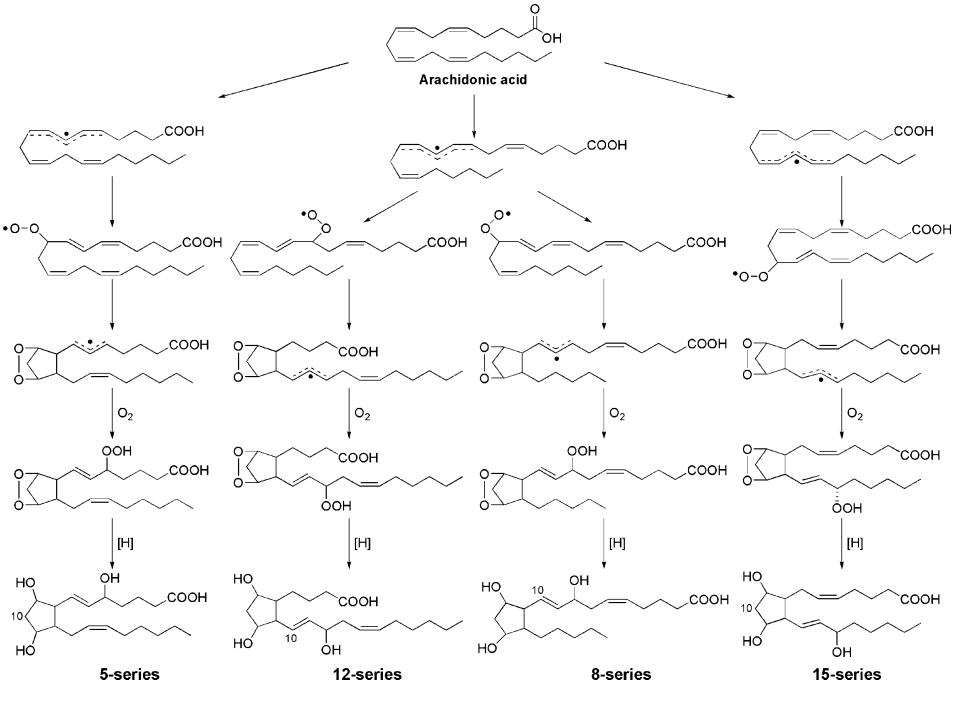

Figure 1. Mechanism of formation of the F_2-IsoPs. Four regioisomers are formed each consisting of eight racemic diastereomers. For simplicity, stereochemistry is not indicated.

structures (such as those resembling PGD_2/E_2 and PGA_2/J_2) can also be formed by this mechanism (Chen et al. 1999, Reich et al. 2001a). F_2-IsoPs, however, have been the most studied class of IsoPs and, because of their stability, afford the most accurate measure of oxidant stress (Liu et al. 1999). An important structural distinction between IsoPs and cyclooxygenase-derived PGs is that the former contain side chains that are predominantly oriented *cis* to the prostane ring while the latter possess exclusively *trans* side chains (Morrow et al. 1990a, b). A second important difference between IsoPs and PGs is that IsoPs are formed *in situ* esterified to phospholipids and are subsequently released by a phospholipase(s) (Liu et al. 1999, Morrow and Roberts 1999), while PGs are generated only from free arachidonic acid.

Quantification of F_2-isoprostanes

Over the past 12 years, several methods have been developed to quantify the F_2-IsoPs. The laboratory uses a gas chromatographic/negative ion chemical ionization mass spectrometric (GC/NICI-MS) approach employing stable isotope dilution (Morrow and Roberts 1999). For quantification purposes, the F_2-IsoP, 15-F_{2t}-IsoP and other F_2-IsoPs that co-elute with this compound are measured. Several internal standards are available from commercial sources to quantify the IsoPs. These assays typically use either [$^2H_4$]15-F_{2t}-IsoP ([$^2H_4$] 8-iso- $PGF_{2\alpha}$) or [$^2H_4$]-$PGF_{2\alpha}$ as internal standards. The advantages of mass spectrometry over other approaches include its high sensitivity and specificity, which yields quantitative results in the low picogram range. Its drawbacks are that it is labour intensive and requires considerable expenditures on equipment.

Several alternative mass spectrometric assays have been developed by different investigators including FitzGerald and colleagues (Rokach et al. 1997, Pratico et al. 1998a). Like this assay, these methods require solid phase extraction using a C18 column, TLC purification and chemical derivitization. Further, IsoPs are quantified using isotope dilution GC/NICI-MS, but the assay measures F_2-IsoP isomers other that 15-F_2-IsoP. These methods appear to be comparable to this one in terms of utility. In addition to these GC/MS assays, a number of liquid chromatographic MS methods for F_2-IsoPs have recently been developed. These methods require less sample preparation (Liang et al. 2000, Bohnstedt et al. 2003), but their sensitivity and reliability for the analysis of IsoPs in complex biological samples is unknown.

Alternative methods have been developed to quantify IsoPs using immunological approaches (Morrow et al. 1999b). Antibodies have been generated against 15-F_{2t}-IsoP and at least three immunoassay kits are commercially available. A potential drawback of these methods is that limited information is currently available regarding their precision and accuracy. In addition, little data exist comparing IsoP levels determined by immunoassay to MS. Analogous to immunological methods to quantify cyclooxygenase-derived PGs, it might be predicted that immunoassays for IsoPs will suffer from a lack of specificity (Roberts and Morrow 2000). Furthermore, the sensitivity and/or specificity of these kits may vary substantially between manufacturers. However, while mass spectrometric methods of IsoP quantification are considered the 'gold standard', immunoassays have expanded research in this area due to their low cost and relative ease of use.

F_2-Isoprostanes as an index of oxidant stress *in vivo*

It has been previously recognized that one of the greatest needs in the field of free radical research is a reliable non-invasive method to assess lipid peroxidation *in vivo* in humans (Roberts and Morrow 2000). In this respect, most methods available to assess oxidant stress, which are adequate for *in vitro* purposes, have suffered from a lack of sensitivity and/or specificity or are unreliable when applied to complex biological fluids and tissues. However, a substantial body of evidence indicates that measurement of F_2-IsoPs in body fluids such as plasma provides a reliable approach to assess lipid peroxidation *in vivo* and represents a major advance in the ability to assess oxidative stress status in animals and humans (Morrow and Roberts 1997, Roberts and Morrow 2000). To this end, this study defined normal levels of F_2-IsoPs in human plasma and urine (Liu et al. 1999, Morrow and Roberts 1999, Morrow et al. 1999b). It is important to note that quantities of these compounds exceed those of cyclooxygenase-derived PGs by at least an order of magnitude, suggesting that IsoPs are a major pathway of arachidonic acid disposition. Furthermore, levels of F_2-IsoPs are sufficient to be detected in every normal biological fluid that has been assayed including plasma, urine, bronchoalveolar lavage fluid, cerebrospinal fluid and bile (Morrow et al. 1999b).

This finding showing the formation of significant levels of F_2-IsoPs in normal human biological fluids and tissues is important for two primary reasons. First, it indicates that there is ongoing lipid peroxidation that is incompletely suppressed by anti-oxidant defenses. This data lends support to the hypothesis that the normal ageing process is due to enhanced oxidant damage of relevant biological molecules over time. In this regard, previous studies have suggested that IsoP levels in normal mice and humans increase with age (Rokach et al. 1997, Roberts and Reckelhoff 2001), although another report refutes this (Feillet-Coudray et al. 1999). Secondly, it suggests that the measurement of F_2-IsoPs in urine can be used as an index systemic or 'whole body' oxidant stress integrated over time. However, the measurement of free F_2-IsoPs in urine can be confounded by the potential contribution of local IsoP production in the kidney, although the extent to which this occurs is unclear (Morrow and Roberts 1997, Morrow et al 1999b, Roberts and Morrow 2000). In light of this issue, this study has identified the primary urinary metabolite of 15-F_{2t}-IsoP to be 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP and has developed a highly sensitive and accurate mass spectrometric assay to quantify this molecule (Roberts et al. 1996, Morrow et al. 1999c, Morales et al. 2001). Thus, the quantification of 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP may represent a truly non-invasive, time-integrated measurement of systemic oxidation status that can be applied to living subjects.

F_2-Isoprostane formation in human disesease

As illustrated in the discussion above, using the GC/NICI-MS methods, one is able to measure normal levels of F_2-IsoP production in healthy subjects in a variety of biological fluids. Thus, it appeared that F_2-IsoP formation could be used to be a reliable index of lipid peroxidation *in vivo* and, thus, potentially provides a tool to assess the role of oxidative stress in the pathophysiology of human disease. Elevations of IsoPs in human body fluids and tissues have been found in a diverse array of human disorders (Table I).

Table I. Disorders in which F_2-IsoPs have implicated a role for free radicals in human diseases.

Disorder	Reference
Acute chest syndrome of sickle cell disease	Klings et al. (2001)
Acute cholestasis	Leo et al. (1997)
Adult respiratory distress syndrome	Carpenter et al. (1998)
Alcohol-induced liver injury	Aleynik et al. (1999)
Allergic asthma	Dworski et al. (1999)
Alzheimer's disease	Montine et al. (1999c)
Chronic obstructive lung disease	Pratico et al. (1999)
Crohn's disease	Wendland et al. (2001)
Diabetes	Gopaul et al. (1995)
Haemodialysis	Spittle et al. (2001)
Hepatorenal syndrome	Morrow et al. (1993)
Huntington's disease	Montine et al. (1999b)
Hypercholesterolemia	Davi et al. (1997a, b)
Hyperhomocysteinemia	Voutilainen et al. (1999)
Interstitial lung disease	Montuschi et al. (1998)
Ischemia/reperfusion injury	Reilly et al. (1997)
Muscular side effects of statins	Sinzinger et al. (2001)
Pulmonary hypertension	Cracowski et al. (2001)
Rhabdomyolysis renal injury	Holt et al. (1999)
Rheumatic inflammatory response	Basu et al. (2001)
Scleroderma	Stein et al. (1996)
Sepsis	Ekmekcioglu et al. (2002)
Smoking	Morrow et al. (1995)

For the purposes of this brief review, it was chosen to discuss IsoP formation in several human diseases in which their generation has been examined in some detail, namely atherosclerosis, Alzheimer's disease and select pulmonary disorders. For a more detailed discussion of these and other disorders, the reader is referred to the cited references in Table I.

Atherosclerotic cardiovascular disease and risk factors for atherosclerosis

The authors and others have extensively explored the association between various risk factors for atherosclerosis and enhanced IsoP generation and have found that IsoP formation is increased in humans with a number of risk factors. These data suggest that enhanced oxidant stress may play a role in the development of this disorder, although the mechanisms involved have not been elucidated. Although trials with antioxidants have generally failed to show benefit in the prevention of heart disease in humans, there is still considerable evidence to support the hypothesis that oxidative stress is intimately involved in the pathogenesis of atherosclerosis.

In accordance with the LDL oxidation hypothesis of atherosclerosis, levels of F_2-IsoPs should be higher in atherosclerotic plaques than in normal vascular tissue. To address this issue, levels of F_2-IsoPs were measured in fresh advanced atherosclerotic plaque tissue removed during arterial thrombarterectomy ($n = 10$) and compared with levels measured in normal human umbilical veins removed from the placenta immediately after delivery ($n = 10$) (Gniwotta et al. 1997). Levels of F_2-IsoPs esterified in vascular tissue normalized to both wet weight and dry weight were significantly higher in atherosclerotic plaques compared to normal vascular tissue. When the data was normalized to arachidonic acid content, the F_2-IsoP/arachidonic

acid ratio was ~ 4-fold higher than the ratio in normal vascular tissue ($p = 0.009$). This finding indicates that unsaturated fatty acids in atherosclerotic plaques are more extensively oxidized than lipids in normal vascular tissue. These observations are also in accord with data from FitzGerald and colleagues who have shown increased amounts of F_2-IsoPs in human atherosclerotic lesions including the localization of F_2-IsoPs in atherosclerotic plaque tissue to foam cells and vascular smooth muscle cells (Pratico et al. 1997).

Atherosclerosis and hypercholesterolemia. It has been well established that patients with hypercholesterolemia have an increased risk for the development of atherosclerosis. Thus, it was of interest to determine whether levels of F_2-IsoPs are increased in patients with this condition. Levels of F_2-IsoPs esterified in plasma lipids were determined in patients with polygenic hypercholesterolemia (Roberts and Morrow 1999). Levels in patients with hypercholesterolemia were found to be significantly increased a mean of 3.4-fold (range 1.7–7.5-fold) above levels measured in normal controls ($p < 0.001$). Interestingly, in these patients, there was no correlation between levels of F_2-IsoPs and serum cholesterol, triglycerides or LDL-cholesterol. In addition, plasma arachidonic acid content was measured in these patients and normal controls. Again, no correlation between IsoP and arachidonate levels was found. Thus, these data suggest that the finding of high levels of F_2-IsoPs in patients with hypercholesterolemia is not due simply to the presence of more lipid, i.e. arachidonic acid substrate. Rather, it is suggested that hypercholesterolemia is associated with enhanced oxidative stress. The underlying basis for this observation, however, remains unclear. Interestingly, a report also found that the urinary excretion of F_2-IsoPs was also increased in patients with Type II hypercholesterolemia by a mean of 2.5-fold, which was suppressed by ~ 60% with vitamin E treatment (600 mg per day) (Davi et al. 1997a, b).

Atherosclerosis and diabetes. Patients with diabetes mellitus are known to have an increased incidence of atherosclerotic vascular disease. Interestingly, the formation of F_2-IsoPs has been found to be induced in vascular smooth muscle cells *in vitro* by elevated glucose concentrations (Natarajan et al. 1996). Thus, a number of investigators have explored whether there is evidence for enhanced oxidative stress *in vivo* in patients with diabetes (Oguogho and Sinzinger 2000, Davi et al. 2004).

F_2-IsoPs have been found to be increased in both Type I and Type II diabetes, although the reason for enhanced lipid peroxidation is not clear. In the case of Type I disease in children and adolescents, increases in IsoP formation represents an early event in the disease and decrease as the disease progresses (Davi et al. 2003). In the case of Type II diabetes, a number of reports have noted increases in plasma and urine F_2-IsoPs in humans. Davi et al. (1999) have reported that urinary IsoP excretion is markedly enhanced in the majority of a large group of patients with Type II diabetes who were carefully characterized for other variables that affect F_2-IsoP formation. They also found a highly significant correlation between blood glucose and urinary IsoP levels suggesting that lipid peroxidation is related to glycemic control. Further, the hypothesis that impaired glycemic control rather than some other factor is responsible for enhanced formation of F_2-IsoPs in Type II disease is also supported by the fact that intensive anti-diabetic treatment induced reductions in both blood glucose levels and in urinary IsoP levels. In addition, increases in platelet activation

induced by hyperglycemia parallelled increases in oxidant stress (Davi et al. 1997a, b). This is of interest since 15-F_{2t}-IsoP is a ligand for the thromboxane receptor (Fam and Morrow 2003).

In addition, this study explored whether there was evidence for enhanced oxidative stress *in vivo* in patients with diabetes (Koulouris et al. 1996). In this study, levels of F_2-IsoPs esterified in plasma lipids were quantified in 61 patients who underwent coronary angiography, 15 of whom had diabetes. The extent of coronary atherosclerosis in the diabetic patients was similar to that in the 46 non-diabetic individuals. Plasma levels of F_2-IsoPs measured in the diabetic patients (33.4 ± 4.8 pg ml^{-1}, mean $\pm$ SEM) were found to be significantly increased compared with levels measured in the non-diabetic patients (22.2 ± 1.9 pg ml^{-1}) ($p < 0.02$). Similar findings have also been reported by Gopaul et al. (1995), in which they found a mean 3.3-fold increase in free F_2-IsoP concentrations in plasma of diabetic patients compared to non-diabetic healthy control subjects. In addition, it has been reported that urinary IsoP levels in diabetics are suppressed by vitamin E and by control of hyperglycemia (Lawson et al. 1999).

Atherosclerosis and obesity. The marked increase in the incidence of overweight and obese persons is recognized as perhaps the most serious public health issue in the US. It is estimated that currently greater than 60% of American adults are overweight and 20% are obese (Eckel et al. 2002). Being overweight or obese is associated with a significantly increased mortality from atherosclerotic cardiovascular disease and other causes (Calle et al. 1999, National Task Force on the Prevention and Treatment of Obesity 2000, Fontaine et al. 2003). Although obesity itself appears to augment the incidence of cardiovascular events, it is also associated with major risk factors for atherosclerosis including hyperlipidemia, diabetes mellitus, hypertension and the metabolic syndrome (Eckel et al. 2002, Grundy 2002). How obesity and each of these risk factors are involved mechanistically in atherosclerosis have been areas of intense research but are poorly understood. Nonetheless, at least three recent reports provide evidence that elevated systemic oxidant stress may be an important mechanism by which obesity increases the incidence of atherosclerotic cardiovascular disease (Block et al. 2002, Davi et al. 2002, Keaney et al. 2003).

In a study by Keaney et al. (2003), it is reported that an association exists between increasing body mass index (BMI) and increasing systemic oxidant stress. Utilizing the quantification of urinary F_2-IsoPs, the authors show in nearly 3000 patients involved in the Framingham Heart Study that enhanced IsoP formation in both men and women is strongly associated with increasing BMI. These findings add support to two smaller studies in which overweight/obesity was associated with enhanced oxidant stress and IsoP formation (Block et al. 2002, Davi et al. 2002). The importance of the work by Keaney is that the study population was not a smaller, targeted one but one involving a large community-based cohort of otherwise healthy individuals. A particularly relevant aspect of Keaney's work with respect to determining the role that obesity-associated oxidant stress plays in atherosclerotic cardiovascular disease is the fact that participants in the trial will be followed over time so that clinical outcomes such as cardiovascular events can be correlated with excessive oxidant stress. In this respect, this study allows for a more direct assessment of the extent to which oxidative injury contributes to atherosclerotic sequelae in humans than do previously reported intervention trials that utilized, for example, anti-oxidants. It

should also be noted that Patrono and colleagues have reported that weight loss in obese women is associated with a significant reduction in IsoP formation (Davi et al. 2002).

Alzheimer's disease

Oxidative stress has been implicated in the pathogenesis of numerous neurodegenerative conditions, including Alzheimer's Disease (AD). Regional increases in oxidative damage and lipid peroxidation have been described in brain tissue obtained post-mortem from patients with AD (Markesbery 1997). Similarly, F_2-IsoP levels are significantly elevated in affected regions of post-mortem brain samples from AD patients as compared to controls (Reich et al. 2001a). However, an objective index of oxidative damage associated with AD that can be assessed in living patients has previously been lacking. Such a biomarker could be vital for understanding the role oxidative damage in AD patients by permitting repeated evaluation of disease progression or responses to therapeutic interventions. Toward such a goal, post-mortem ventricular fluid was obtained from 11 patients with a pathological diagnosis of AD and 11 control patients, in order to evaluate F_2-IsoP levels in cerebrospinal fluid (CSF) (Montine et al. 1998). All subjects participated in a rapid autopsy protocol such that fluid was collected within 3 hours of death. F_2-IsoP levels were significantly increased in ventricular fluid from AD patients (72 ± 7 pg ml^{-1}, mean $\pm$ SEM) compared to CSF from control individuals (46 ± 4 pg ml^{-1}, $p < 0.01$) and correlations were identified between increases in IsoP levels and higher Braak stage and decreased brain weight, two indices of AD severity. A larger study has shown that CSF F_2-IsoP level correlates with the extent of pathological neurodegeneration but not with density of neuritic plaques or neurofibrillary tangles (Montine et al. 1999c, Reich et al. 2001b).

Subsequently, a study was undertaken to examine CSF F_2-IsoP levels in living patients with probable AD (Montine et al. 1999a). CSF was obtained from the lumbar cistern in 27 patients with AD and 25 controls without neurodegenerative disorders matched for age and gender. In keeping with post-mortem studies, lumbar CSF levels of F_2-IsoPs were significantly increased (31.0 ± 2.6 pg ml^{-1}) in AD patients compared to control subjects (22.9 ± 1.0 pg ml^{-1}, $p < 0.05$). Pratico et al. (2000) have also observed increased F_2-IsoPs in CSF of patients with probable AD, as well as in patients with mild cognitive impairment (MCI), a condition which precedes symptomatic dementia in AD (Pratico et al. 2002). However, this group also reports increased F_2-IsoPs in plasma and urine of both MCI and AD patients, although the laboratory and others have not been able to detect these changes in peripheral F_2-IsoPs (Montine et al. 2002, Bohnstedt et al. 2003). Taken together, these studies suggest that quantification of IsoPs in cerebrospinal fluid of patients with Alzheimer's disease may be of use as an *intra-vitum* index of disease progression or as a tool to monitor response to therapy.

Pulmonary disease

The interest in the quantification of IsoPs as an index of oxidative injury in human pulmonary disease stems, in part, from studies that have explored the biological activities of these compounds in the pulmonary circulation and the bronchial tree. The physiological effects of IsoPs in the lung have been recently discussed in an

outstanding review by Janssen (2001). As he notes, these compounds are likely not only markers of oxidant stress but play a role in pulmonary pathophysiology since they evoke important biological responses on most cell types in the lung. Further, they exhibit differences with respect to the various species and tissues studied. The two isoPs examined in greatest detail in the lung are 15-F_{2t}-IsoP and 15-E_{2t}-IsoP (Janssen 2001). In general, these compounds are potent constrictors of pulmonary vascular smooth muscle and airway smooth muscle. Effects are mediated by interactions with the thromboxane receptor, a G-protein coupled eicosanoid receptor, and potentially other eicosanoid receptors (Morrow and Roberts 1997, Janssen 2001). Subsequently, they induce various intra-cellular second messenger systems including phospholipase C/inositol trisphosphate and mitogen-activated protein (MAP) kinase that result in constriction (Fukunaga et al. 1993a, b Fukunaga et al. 1997). Further, IsoPs activate various inflammatory cells such as neutrophils leading to enhanced adhesion to endothelial cells. The increase in adhesion, however, occurs independently of increased expression of various adhesion molecules (Zahler and Becker 1999). Following is a brief discussion of IsoP formation in several pulmonary disorders.

Cigarette smoking. A link between cigarette smoking and risk of pulmonary and cardiovascular disease is well established. However, the underlying mechanism(s) for this effect is not fully understood. The gaseous phase of cigarette smoke contains a number of oxidants and exposure of the lung to the gaseous phase of cigarette smoke *in vitro* induces oxidation of tissue and circulating lipids (Frei et al. 1991). Thus, the hypothesis was explored that smoking induces an oxidative stress and specifically determined whether circulating plasma lipids in individuals who smoke contain higher levels of F_2-IsoPs, indicative of a greater degree of oxidative modification. Ten individuals who smoked heavily ($>$ 30 cigarettes per day) and 10 age- and sex-matched non-smoking normal volunteers were studied (Morrow et al. 1995). Plasma concentrations of free and esterified F_2-IsoPs were significantly elevated in the smokers compared to the non-smokers ($p = 0.02$ and $p = 0.03$, respectively). Confirmation that these differences in levels of F_2-IsoPs between smokers and non-smokers were due to cigarette smoking was obtained by measuring levels of F_2-IsoPs following 2 weeks of abstinence from smoking in eight of the 10 smokers who successfully abstained. In all subjects, levels of F_2-IsoPs both free in the circulation and esterified to plasma lipoproteins were significantly lower following 2 weeks of abstinence from smoking ($p = 0.03$ and $p = 0.02$, respectively). The occurrence of enhanced formation of IsoPs in smokers has subsequently been confirmed in studies by others (Reilly et al. 1996, Obwegeser et al. 1999). Collectively, these findings suggest strongly that smoking causes an oxidative stress and the observation that smokers have elevated levels of F_2-IsoPs esterified in plasma lipids also supports the hypothesis that the link between smoking and risk of pulmonary and cardiovascular disease may be attributed to enhanced oxidation of tissue and or circulating lipids.

Allergen-induced asthma. Asthma is a chronic inflammatory disease of the airways that is believed to involve oxidant injury to the lung, although firm evidence to support this contention was lacking. Recently, a series of studies was undertaken to quantify F_2-IsoP formation in a group of 11 patients with mild atopic asthma after an inhaled allergen challenge (Dworski et al. 1999). The urinary excretion of F_2-IsoPs increased significantly by a mean of 73% at 2 hours after allergen challenge and remained

significantly elevated in all urine collections for the 8-hour period of the study. Urinary IsoPs did not change after inhaled methacholine challenge. In nine of the atopic patients, F_2-IsoPs were quantified in bronchoalveolar lavage fluid at baseline and 24 hours after allergen installation. F_2-IsoPs were significantly elevated (mean 27%) late in the lavage fluid. Subsequently, one has also shown significant increases in excretion of the the major urinary metabolite of 15-F_{2t}-IsoP in atopic asthmatics after allergen exposure (Dworski et al. 2001). These findings have been confirmed by other investigators (Wood et al. 2000). In addition, Barnes and colleagues have also shown that IsoP levels are significantly increased in exhaled breath condensate from asthmatics compared healthy humans (Montuschi et al. 1999). This study is particularly interesting because it suggests that non-invasive methods such as breath analysis can be utilized to detect oxidative injury locally in pulmonary tissues. Overall, these observations are extremely important in that they provide new evidence for a role of oxidant stress and lipid peroxidation in allergen-induced airway inflammation and, thus, identify a potential therapeutic target for the treatment of this common pulmonary disorder.

Conclusions

The discovery of IsoPs as products of non-enzymatic lipid peroxidation has been a major breakthrough regarding the quantification of oxidant stress *in vivo*. The quantification of these molecules has opened up new areas of investigation regarding the role of free radicals in human physiology and pathophysiology and appears to be the most useful tool currently available to explore the role of lipid peroxidation in the pathogenesis of human disease. Although considerable information has been obtained since the initial discovery of IsoPs, much remains to be understood about the role of these molecules as markers of oxidant stress *in vivo*. It is anticipated that additional research in this area will continue to provide important insights into the role of oxidative stress in human disease.

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