Oxidative Stress and Vascular Disease: Insights from Isoprostane Measurement

Over the last 20 years, overwhelming evidence has accumulated indicating that oxidation of lipoproteins plays an important role in the development of atherosclerosis (1). The pathophysiology of atherosclerosis is characterized by two key processes, lipid deposition and inflammation, within the arterial wall, and oxidative stress provides a link between the two (2). Despite general acceptance of the importance of oxidative events in atherosclerosis, clinical trials of antioxidant supplementation have produced disappointing results (3–5). There may be several explanations for this, including use of inappropriate antioxidants or antioxidant combinations, incorrect doses, insufficient duration of treatment, or failure to initiate treatment sufficiently early in the atherosclerotic process. Alternatively, the apparent association of oxidative stress and atherosclerosis may be an epiphenomenon.

In this issue of the Journal, Gross et al. (6) report an association between increased concentrations of circulating F2-isoprostanes and coronary artery calcification (CAC) in healthy young adults participating in the CARDIA study. Plasma F2-isoprostanes were associated with CAC in both men and women independently of conventional cardiovascular risk factors and C-reactive protein. CAC is a strong predictor of vascular events in prospective studies and can be considered as a good intermediate marker of vascular disease. These findings, therefore, confirm an association between oxidative damage and the early stages of atherosclerosis in humans and support the hypothesis that oxidative damage is involved in the early development of atherosclerosis.

Difficulties in accurately assessing oxidative stress in biological systems have considerably handicapped the investigation of oxidative stress in disease. Numerous tests have been used in clinical studies. In general terms, these can be divided into markers of oxidative damage (to lipids, protein, and DNA) and of antioxidant status (either individual antioxidants or total antioxidant capacity). Often the correlation between any two markers of oxidative stress is poor. In recent years, the measurement of isoprostanes in plasma or urine has emerged as the best marker of oxidative stress (7, 8).

Isoprostanes are stable end-products of lipid peroxidation derived from arachidonic acid. Non-radical generation of isoprostanes in vivo is generally considered to be trivial. A large number of end-products can theoretically be generated, but most interest has focused on the F2-isoprostanes and, in particular, on 8-iso-prostaglandin F2α (PGF2α). In the human circulation, isoprostanes are present mainly in their ester forms, whereas only hydrolyzed isoprostanes are excreted in the urine. The presence of detectable concentrations of isoprostanes in biological fluids implies continuing lipid peroxidation despite the presence of a complex network of antioxidant defenses. As well as being the best available marker of oxidative stress, some isoprostanes have biological activities that may be relevant to the pathophysiology of disease, acting, for example, as broncho- and vasoconstrictors (9, 10).

Various approaches are available for the measurement of F2-isoprostanes, including gas chromatography–mass spectrometry (GC-MS), GC–tandem MS, liquid chromatography–tandem MS, and immunoassays (11). Immunoassay results for 8-iso-PGF2α correlate reasonably with GC-MS measurement in urine, but discrepancies may occur in the case of plasma measurements, perhaps reflecting cross-reactivity with other prostaglandin metabolites. At present, there is no widespread consensus as to the best methodology for measurement, but chromatographic methods should be viewed as superior to immunoassays.

Increased F2-isoprostanes are associated with the presence of the main cardiovascular risk factors. Urinary and plasma isoprostanes are increased in patients with hypercholesterolemia (12). A relatively weak positive association also exists between urinary excretion of isoprostanes and hypertension (13). Plasma and urinary isoprostanes are increased in both type 1 and type 2 diabetes (14, 15) and are reduced by an improvement in glycemic control. Given the documented increase of isoprostanes in type 2 diabetes, it is not surprising that there is a graded relationship with body mass index (16). Cigarette smoking represents a strong oxidant stress, and numerous studies have shown that both urinary and plasma isoprostanes are increased in smokers and that they return to baseline within several weeks of stopping smoking (17–19). In addition to their association with cardiovascular risk factors, isoprostanes are also increased in patients with established vascular disease (20). Although isoprostanes have been demonstrated in atherosclerotic plaques (21), increased serum concentrations and urinary excretion rates are more likely to reflect a systemic increase in oxidative stress than leakage of isoprostanes from plaque into the circulation.

Supplementation with antioxidant vitamins is known to increase antioxidant capacity and to decrease markers of oxidative stress. Interestingly, however, and in contrast to most other biochemical markers of oxidative stress, studies assessing the effects of supplementation with various antioxidants on isoprostanes have produced inconsistent results (11), although benefits are more likely in individuals subject to increased ongoing oxidative stress (15, 17). This may be relevant to the failure of antioxidant supplements to prevent vascular events in clinical trials.

Despite extensive recognition of the importance of oxidative stress in cardiovascular and other disease, measurement of markers of oxidative damage or antioxidant status have not entered the clinical arena. In part, this has been a consequence of recognition of the limitations of biochemical markers. The development of isoprostane analysis represents a considerable advance on previously available tests, but measurement is still restricted to a
research setting, and further evidence will be required if isoprostane measurement is to enter clinical use. What is needed for measurements of isoprostanes to enter the clinical arena? First, there is a need for prospective data showing that increased plasma or urinary concentrations of isoprostanes predict cardiovascular outcomes or other disease. Studies addressing this are in progress; if isoprostanes can be shown to provide additional prognostic information compared with other biomarkers and to significantly improve risk assessment, then clinical use will be more likely. Secondly, improved standardization of analytical methodology is needed. Although immunoassays would be most convenient for most clinical laboratories, at present chromatographic techniques appear superior. Finally, there is also a need for increased understanding of isoprostane physiology, including biological variability and the influence of environmental factors and disease.

While these data accumulate, isoprostane measurement in a research setting can continue to illuminate our understanding of disease. Studies such those of Gross et al. (6) indicate that it is premature to reject an important role for oxidative stress in atherosclerosis and that increased emphasis on lifestyle interventions that reduce oxidative stress in early life may yet pay dividends in the form of reduced vascular disease in maturity.

References

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