Invited Review

THE ISOPROSTANES: UNIQUE BIOACTIVE PRODUCTS OF LIPID PEROXIDATION

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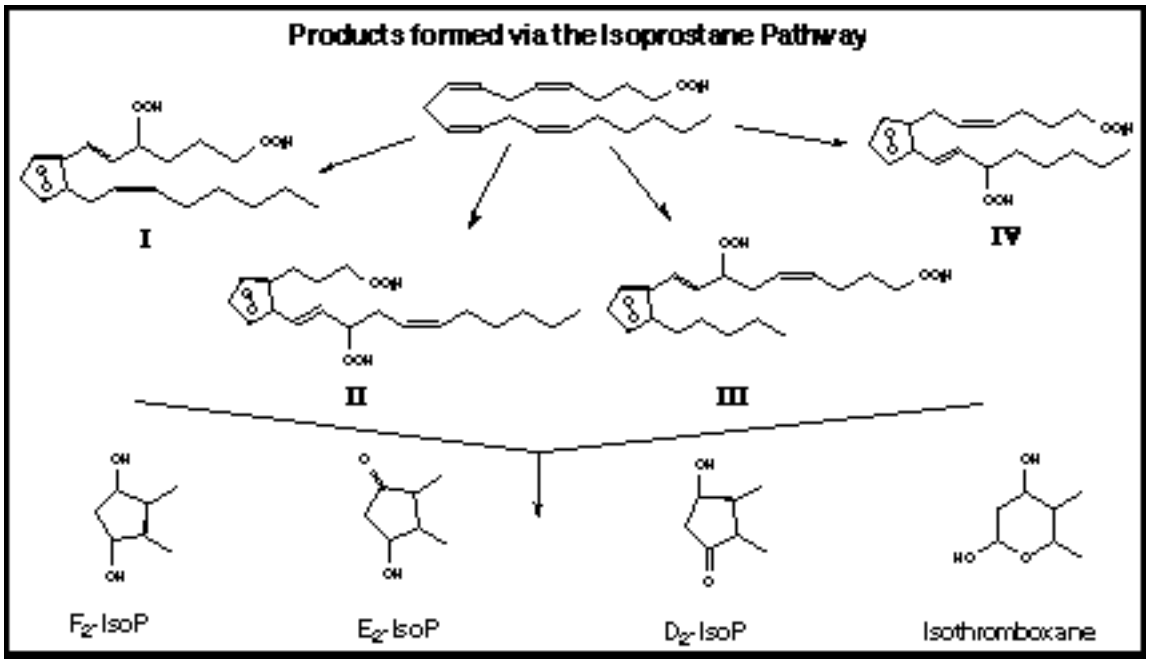
In 1990, we reported the discovery that prostaglandin (PG) F2-like compounds are formed in abundance *in vivo* by free radical catalyzed peroxidation of arachidonic acid, independent of the cyclooxygenase enzyme (1). Since these compounds are isomeric to prostaglandin F2_ formed by the cyclooxygenase, they have been termed F2-isoprostanes (F2-IsoP's). Intermediates in the formation of the IsoP's are bicyclic PGG2-like endoperoxides. As summarized in the Figure below, in addition to the reduction of the IsoP endoperoxides to form F2-IsoP's, they also undergo rearrangement *in vivo* to form PGD2-like compounds (D2-IsoP's), PGE2-like compounds (E2-IsoP's), and thromboxane (Tx)-like compounds (IsoTx's) (2,3).



A unique feature of the IsoP's and IsoTx's is that, unlike eicosanoids derived from the cyclooxygenase, they are initially formed *in situ* on phospholipids and subsequently released preformed (4). Another notable feature of IsoP's and IsoTx's that distinguishes these compounds from eicosanoids derived from the cyclooxygenase is that the side chains of IsoP's are predominantly oriented *cis* in relationship to the cyclopentane ring (1). Thus, we hypothesized that one of the F2-IsoP's that would be produced would be 8-iso-PGF2 alpha. Recently, we confirmed that 8-iso-PGF2 alpha is in fact one of the F2-IsoP's that is formed *in vivo* (5).

Since 8-iso-PGF2 alpha is one of the IsoP's formed *in vivo*, it was of potential importance to explore whether 8-iso-PGF2 alpha exerted biological activity. 8-iso-PGF2 alpha was found to be a very potent renal and pulmonary artery vasoconstrictor (1,6-8). It has also been shown to induce mitogenesis in vascular smooth muscle cells and induce endothelin release from endothelial cells (9,10). Interestingly, we have also found that 8-iso-PGE2 is also a potent vasoconstrictor (2). Although the vascular actions of 8-iso-PGF2_ and 8-iso-PGE2 can be abrogated by SQ29548, a thromboxane receptor antagonist, indirect evidence has

been obtained suggesting the interesting possibility that both 8-iso-PGF2 alpha and 8-iso-PGE2 may exert their vascular effects by interacting with a novel "isoprostane" receptor (6,9-13).

One of the greatest needs in the field of free radical research has been the availability of a reliable method to assess oxidative stress status *in vivo* (14). In this regard, we have accumulated a substantial body of evidence which suggests strongly that measurement of IsoP's represents an important advance in this area (15,16). We have demonstrated that measurement of IsoP's correlates well with other measures of lipid peroxidation *in vitro*, *e.g*. with malondialdehyde and lipid peroxides (17,18). However, measurement of IsoP's appears to represent a more sensitive marker of lipid peroxidation *in* vivo and circumvents other problems, *e.g*. lack of specificity, associated with some of the other methods available to assess lipid peroxidation (14,17,19).

Our laboratory originally developed methodology based on stable isotope dilution techniques utilizing gas chromatography/mass spectrometry for measurement of the array of F2-IsoP's (20,21). Others have also developed mass spectrometric methods for the specific measurement of the F2-IsoP, 8-iso-PGF2 alpha (22). Mass spectrometric methodology for measurement of IsoP's is highly specific and sensitive. However, compared to other methods of assay, *e.g*. immunoassay, it is more labor intensive, has a lower sample throughput, and requires expensive instrumentation, which limits its use widely. Thus, the development of immunoassays for F2-IsoP's would facilitate the use of measurements of F2-IsoP's by investigators in free radical research to assess oxidant injury.

Immunoassays for the F2-isoprostane, 8-iso-PGF2_, have been developed by Oxford Biomedical Research and others (22). In addition, a recent report utilized immunopurification with quantitation by mass spectrometry for measurement of 8-iso-PGF2_ (23). As with other immunoassays for eicosanoids, purification of the eicosanoid from biological fluids prior to assay is frequently necessary to avoid interference from biological substances. This may involve simple extractions or more extensive purification using TLC or HPLC.

In summary, measurement of IsoP's appears to be an important advance in our ability to assess oxidant injury, particularly *in vivo*. Although mass spectrometric methods for analysis of IsoP's are highly specific and sensitive, they are not generally available. However, the use of validated immunoassays for IsoP's should allow the more general use of this approach to detect lipid peroxidation *in vitro* and assess oxidative stress status *in vivo* to explore the role of free radicals in human disease processes.

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