

EDITORIAL

Urinary F_2 -isoprostane metabolite analysis: a step closer to obtaining a reliable measure of oxidative stress?

Oxidative stress, the consequence of an unequal balance between reactive oxygen species (ROS) production and antioxidant defences has been implicated in a wide range of diseases, including those of the respiratory system. In its most severe form, redox imbalance can result in cell death following widespread macromolecule oxidation while more subtle redox changes appear to play a role in modulating a range of signal transduction pathways [1]. All molecules are potential targets for ROS, but because of their ubiquitous distribution within cell membranes and their propensity to contain double bonds, unsaturated lipids are often targeted. Indeed, investigators determining the impact of the oxidation of cell lipids drove the massive growth in free radical biology during the 1960s and 1970s. Unfortunately, most of the early methods used to assess lipid oxidation were non-specific, inaccurate and gave variable results in different laboratories. As a result of these limitations, their application in clinical studies provided little major advancement in knowledge.

During the 1980s and 1990s methodological advances gave rise to more accurate ways to assess the extent of lipid peroxidation occurring *in vivo*. These included the measurement of malondialdehyde and lipid hydroperoxide content of biological samples by hplc [2]. In 1990, Morrow, Roberts and co-workers provided a further advance by reporting the discovery of prostaglandin F_2 -like compounds, the F_2 -isoprostanes [3]. Isoprostanes are formed by direct ROS attack of arachidonic acid, an unsaturated fatty acid component of cell membranes and low density lipoprotein (LDL). Unlike prostaglandins, enzymatic products of this fatty acid, isoprostanes are formed initially *in situ* in cell membranes from which they are subsequently cleaved by phospholipase(s). Although up to 64 different isoprostanes can arise from the oxidation of arachidonic acid, one of the most stable products is 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$). As a consequence it is this isoprostane which has received most attention in human studies. Perhaps as the field was in such need of a reliable and meaningful measure of *in vivo* lipid peroxidation, 8-iso-PGF $_{2\alpha}$ was rapidly adopted by many as a 'gold standard' marker. Although a little premature in this respect, determining 8-iso-PGF $_{2\alpha}$ concentrations does have several advantages over many existing methods used to access

oxidative stress. For example, 8-iso-PGF $_{2\alpha}$ is both a specific and stable product of lipid peroxidation. Moreover, the quantities of 8-iso-PGF $_{2\alpha}$ increase markedly in a number of animal models of oxidant injury and this can be modified by antioxidant status [3,4].

In humans, most 8-iso-PGF $_{2\alpha}$ analyses have been undertaken using blood and urine samples. Measurements in blood provide an index of isoprostane formation over a relatively short period as their half-life in this compartment is only 20 min [5]. Moreover, because blood contains substantial quantities of arachidonic acid, the substrate for 8-iso-PGF $_{2\alpha}$, there is always the potential of artificial generation of 8-iso-PGF $_{2\alpha}$ through autoxidation, if the samples are not processed and stored properly. Measurements of 8-iso-PGF $_{2\alpha}$ in urine partially circumvent this problem but worries about potential contributions of local formation of isoprostanes in the kidney also exist [6]. As the urinary excretion of prostaglandin metabolites was the most reliable measure of total endogenous prostanoid production, efforts have been directed at developing a technique to measure metabolites of 8-iso-PGF $_{2\alpha}$ in urine. Following such an approach, Roberts, Morrow and co-workers identified a single predominant metabolite of 8-iso-PGF $_{2\alpha}$ in urine [7]. The metabolite, identified by mass spectrometer analysis as 2,3-dinor-5,6-dihydro-15- F_{2t} -IsoP (15- F_{2t} -IsoP-M) is present at baseline concentrations of 0.39 ± 0.18 ng/mg creatinine. Moreover, this level is increased 2.5-fold in patients with polygenic hypercholesterolemia and can be suppressed 54% following 8 weeks treatment with a combination of vitamin E, C and β -carotene [8].

Although now widely utilized in a number of fields, isoprostane analysis has only recently been undertaken in respiratory research. In the last 2 years studies by Barnes and colleagues have demonstrated that 8-iso-PGF $_{2\alpha}$ concentrations are increased in breath condensate of patients with asthma [9], COPD and cystic fibrosis [10]. Although encouraging, these data obtained with use of a commercial ELISA kit for 8-iso-PGF $_{2\alpha}$ need confirmation, as urinary analysis of 8-iso-PGF $_{2\alpha}$ by ELISA and mass spectrometry have revealed considerable inconsistencies [11]. Even so while we wait for confirmation, such findings help support long-held suspicions of many investigators that oxidative

stress plays an important role in inflammatory respiratory diseases. These suspicions are taken a further step forward by the study of Dworski and colleagues in this issue of *Clinical and Experimental Allergy* in which they report that the urinary excretion of 15- F_{2t} -IsoP-M is significantly increased 2 h post-allergen challenge in mild atopic asthmatics [12].

Although these findings can only be considered preliminary (no comparison with control subjects; only nine subjects considered, subjects followed for 8 h only, parallel BAL fluid samples not assessed), they have been made with a new robust marker of oxidative stress and hence, are very encouraging. Moreover, the observation that inhaled provocation with methacholine, a non-specific bronchoconstrictive agent, does not modify 15- F_{2t} -IsoP-M output suggests that its increased production is specific to an allergen-induced asthmatic reaction. If correct, as 15- F_{2t} -IsoP-M arises directly from ROS-mediated activity, it strongly supports the concept of ROS involvement in allergen-provoked asthma. This finding, in turn, helps provide new clues regarding the antioxidant imbalances which exist in asthmatic airways [13] and why there is a rapid loss of superoxide dismutase activity in asthmatic airways following allergen challenge [14]. The current findings of Dworski and colleagues do not provide information on the site(s) of oxidant stress but given that they previously found elevated 8-iso-PGF $_{2\alpha}$ in BAL fluid in a similar setting [15], strongly suggest that the lung is the likely source of the parent molecule. The actual site(s) of metabolic conversion of 8-iso-PGF $_{2\alpha}$ to 15- F_{2t} -IsoP-M are unknown. Hence, analysis of BAL fluid may help resolve if any conversion is occurring within the lung itself.

Although there is a considerable amount of circumstantial evidence implicating oxidative stress in the pathogenesis of many inflammatory lung diseases the hypothesis remains to be proven. As highlighted by Dworski and colleagues, the accurate assessment of airway oxidative stress is an important requirement for investigating the mechanisms underlying many pulmonary diseases [12]. Moreover, it is also important when assessing the benefit of anti-inflammatory and novel antioxidant therapies in such conditions. The availability of new specific techniques, such as that reported in this issue of the journal, is crucial for the advancement of this area of respiratory medicine.

References

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FRANK J. KELLY

Lung Biology

The Rayne Institute

St Thomas' Hospital

King's College

London SE1 7EH

UK

E-mail: frank.kelly@kcl.ac.uk