COMMENT

More knocks to the oxidation hypothesis for vascular disease?

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ABSTRACT

The oxidation hypothesis for CHD (coronary heart disease) is attractive; however, the almost universal failure of antioxidant vitamin supplementation as a CVD (cardiovascular disease) risk modifier challenges the oxidation hypothesis, at least as a concept that easily ‘translates’ into clinical benefit for the population. At the same time, quality prospective data on lipid or protein oxidation markers as predictors of vascular events are sparse. In the present issue of Clinical Science, Woodward and co-workers provide much needed prospective data examining the relationship between markers of oxidative damage and CHD outcome in a general population. Despite noting the expected associations between several established CHD risk factors and CHD events, no significant link was observed between measured oxidation markers and CHD risk, a finding which further challenges the oxidation hypothesis for CHD.

Healthy human physiology relies on a complex balance of pro- and anti-oxidant mechanisms. ROS (reactive oxygen species), such as superoxide anions, free radicals and H₂O₂, are generated as by-products of aerobic metabolism. These pro-oxidant systems are balanced by a battery of antioxidants such as exogenously derived ascorbic acid and endogenous SOD (superoxide dismutase). Pro-oxidant effects are essential for normal processes involved in cell apoptosis and innate immune responses to infection. When oxidative stress occurs in excess and overwhelms antioxidant reserves, lipids, proteins and DNA are indiscriminately damaged, often rendering them dysfunctional and contributing to the development of chronic disease.

A central role for oxidative stress in CVD (cardiovascular disease) is an attractive theory, particularly given that antioxidant administration (in the form of ‘simple and safe’ vitamin supplements) might reverse or at least slow this process in some way. Prior consistent epidemiological evidence of low vitamin levels in subjects at risk of CVD has fuelled an explosion of end-point trials investigating the cardioprotective potential of vitamin supplements. Antioxidant supplementation has, however, generally failed to demonstrate any beneficial effect. A recent Cochrane meta-analysis of 77 randomized trials involving 232,550 participants concluded that there was no evidence to support the use of antioxidants in primary or secondary CVD prevention and, in fact, suggested a potentially deleterious effect with vitamin A, β-carotene and vitamin E [1]. Similarly, observational studies have linked circulating homocysteine levels with the risk of CVD; however, despite successfully lowering homocysteine levels, interventional studies of folic acid and cyanocobalamin have not shown any reduction in CVD risk [2,3]. This illustrates an important concept: attempting to reverse the pathophysiological mechanisms linked to apparently aberrant biomarker levels (in this case, low antioxidant levels) does not necessarily translate into improved outcomes.

The almost universal failure of antioxidant vitamin supplementation as a CVD risk modifier certainly threatens the oxidation hypothesis, at least as a concept...
that translates into clinical benefit for the population. However, a number of considerations have been proposed in this area to try to explain past failures and to help improve future research. The Cochrane review [1] suggests that trial methodologies in the antioxidant field have generally been suboptimal: patient groups varied dramatically with no estimation of baseline oxidative stress levels; there was uncertainty regarding baseline and cardiovascular health; and the risk of bias was high. The interventional supplements also varied massively in terms of dose, duration of treatment and the combinations used. Of course, the results of the antioxidant RCTs (randomized controlled trials) have to be taken at face value; there is currently no role for supplements of naturally occurring antioxidants in the prevention of CVD. With this in mind, it is of interest that Lawlor and co-workers [4] showed that socio-economic factors may confound the relationship between low vitamin levels and CVD risk. In other words, low vitamin levels may not be causally linked to CVD risk; rather they are simply markers of some other more relevant process also linked to socio-economic status.

With the above body of evidence in mind, there is clearly a need for parallel prospective studies linking oxidative stress markers measured at baseline to incident CVD outcomes. But which markers are best and how easy are they to measure? An ever increasing number of biomarkers of oxidative stress are emerging, relying on identification of in vivo lipid and protein targets for oxidation (reviewed in [5,6]). F2-Isoprostanes, derived from non-enzymatic free-radical-mediated peroxidation of arachidonic acid, are arguably the best established biomarker of oxidative stress. In relation to CVD, they have been found to be significantly increased in atherosclerotic plaques compared with healthy non-atherosclerotic vascular tissue; systemic F2-isoprostanes have also been found to increase following angioplasty. The relationship between oxLDL (oxidized low-density lipoprotein) and CVD has also been extensively studied, and there is some evidence, albeit mixed, for oxLDL as an in vivo marker of oxidative stress. Unregulated uptake of oxLDL by scavenger receptors on macrophages results in the formation of foam cells, precursors of advanced atherosclerotic plaque formation. oxLDL is also thought to (i) enhance interactions between monocytes and endothelial cells to promote atherogenesis and plaque instability, (ii) induce the RAS (renin–angiotensin system), and (iii) promote a cascade of inflammatory cytokines, such as IL-6 (interleukin-6) and TNF-α (tumour necrosis factor-α). Finally, HDL (high-density lipoprotein) particles are another target for oxidative stress, and it is possible to measure oxidized apo (apolipoprotein) A-I and apo-A-II as markers for in vivo HDL-cholesterol oxidation. The role of oxHDL (oxidized HDL) in atherogenesis is, however, less clear. Studies have suggested that oxidation of methionine residues on apo-A-I impairs reverse cholesterol transport; however, studies in mice suggest that tyrosyl-radical oxHDL may be more effective at extracting cholesterol [7].

Measurement of markers of oxidative stress is therefore complex and also requires careful sample collection and storage, and invariably labour-intensive methodology, such as HPLC for oxHDL or MS for F2-isoprostanes, as described by Woodward et al. [8]. Other methods involve immunoassays to oxidized moieties of oxLDL. However, there are lingering concerns over the specificity of some ELISA-based methods, as well as uncertainty about the pathophysiological relevance of some oxidative markers measured in the circulation compared with tissue levels (where levels may change in opposite directions). Accurate assessment or interpretation of oxidative stress markers in large-scale population-based studies is therefore not easy and, as a result, there are few robust studies prospectively linking oxidative biomarkers with CVD incident end points. Small-scale studies have suggested a role for F2-isoprostanes (measured in plasma or urine) as independent risk markers for atherosclerosis [9,10]; however, larger prospective studies, with proper adjustment for confounding factors, are noticeably sparse [6].

In the present issue of Clinical Science, Woodward and co-workers [8] report a new prospective study in this area. They measured F2-isoprostanes and oxidized moieties on apoA-I and apoA-II, HDL-related proteins, in a nested case-control study with 227 coronary cases and 420 controls. The authors [8] observed no significant association between the markers measured and CVD event risk. Whether this study had suboptimal power to detect associations between oxidation markers and CHD (coronary heart disease) risk is debatable, as established risk predictors were significantly related to outcomes. In short, the results are disappointing with regards to the oxidation hypothesis. There are little other findings on oxHDL moieties available for comparison, but other markers of oxidation, for example oxidized phospholipids/apoB measurement (an antibody-based assay), have been shown by others to be predictive of 10-year CVD events in a prospective population-based study of 765 subjects [11]. To advance this field, careful collaboration between different groups is required to generate greater power in prospective studies of a range of oxidative markers. Such studies should also employ, wherever possible, gold standard or at least standardized laboratory methods to measure oxidative stress so that results are more robust.

In summary, although the oxidation hypothesis for vascular disease is attractive, there is currently inconsistent evidence linking markers of oxidative stress with incident CVD events. Equally, RCTs of vitamins have failed to show vascular benefit and, in some cases, have suggested possible harm. That said, a recent RCT with a synthetic antioxidant, succinobucol [12], did show some promise in terms of vascular and diabetes risk reduction, although the trial failed to meet its primary end point and
reported some unexpected harmful effects. Given these uncertainties, the foundations of CVD risk prediction should continue with established risk factors, such as lipids, blood pressure and smoking, to determine risk and proven interventions to lessen it.

REFERENCES


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