

Are we over oxidized? Oxidative stress, cardiovascular disease, and the future of intervention studies with antioxidants

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A number of recent clinical trials with antioxidants, notably vitamin C and E, have provided no support for the commonly held view that increasing our intake of antioxidants will offset the ravages of cardiovascular disease as well as other diseases (for extensive critical reviews see: Kritharides and Stocker 2002; Antoniadis et al 2003; Touyz 2004). Is this conclusion justified? The role of antioxidant dietary adjuncts and therapy in prevention and treatment remains a highly important clinical question. In this opinion article we address the question: Is there a future for antioxidant therapy in the treatment and prevention of cardiovascular disease? We conclude that there is a need for better-designed studies as well as a re-thinking of the choice of antioxidants.

What is oxidative stress?

There is considerable literature that indicates that the excessive production of reactive oxygen species (ROS) leads to oxidative stress and the oxidation of biological macromolecules. Oxidative stress is defined as an increase in ROS and/or a decrease in the antioxidant defence mechanisms. Endogenous antioxidants include glutathione peroxidase and CuZn superoxide dismutase (CuZnSOD). Oxidative stress is an important contributory factor to the etiology of many cardiovascular diseases, including atherosclerosis, diabetes, heart failure, and hypertension. An important target for ROS in the pathological cascade is the endothelium, and endothelial dysfunction is increasingly being recognized as an important indicator of the health of the cardiovascular system (Dusting and Macdonald 1995; Cai and Harrison 2000; Verma and Anderson 2002; Triggle

et al 2003). It has been said, “a man is only as old as his endothelium” (Ding and Triggle 2005, p 57).

What do we understand by ROS?

ROS are a family of molecules, including molecular oxygen and its derivatives, which are produced in all aerobic cells through a variety of enzymic processes (Ellis and Triggle 2003; Jiang et al 2004). Many species of ROS possess unpaired electrons and thus are free radicals – these include superoxide anion ($\bullet\text{O}_2^-$), hydroxyl radical ($\bullet\text{OH}$), and the free radical form of nitric oxide ($\bullet\text{NO}$). Other members of the ROS family include hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO^-). Although the important biological functions of NO are well known, it is also now increasingly being realized that other ROS also contribute to physiological and cell signaling pathways (for review see Dröge 2002).

Why does an elevation of ROS lead to cardiovascular disease?

The overproduction of $\bullet\text{O}_2^-$ will be detrimental because of the rapid interaction of $\bullet\text{O}_2^-$ with nitric oxide (NO), which leads to the loss of NO bioavailability, an increase in the production of peroxynitrite (ONOO^-), a subsequent reduction in the vascular effects of NO, as well as a reduction in the antiatherogenic effects of NO. Oxidation of low-density lipoprotein (LDL) leads to lipid peroxidation, which is a major contributor to atherosclerosis and cardiovascular dysfunction. An elevation of $\bullet\text{O}_2^-$ will also lead to the oxidation of the important co-factor in the regulation of nitric oxide synthase, tetrahydrobiopterin (BH_4), and this will lead to an “uncoupled eNOS”, which will then synthesize $\bullet\text{O}_2^-$ rather than NO (Pannirselvam et al 2003; Alp and Channon 2004). *Clearly, if the level of NO and its bioavailability are reduced, cardiovascular function will be compromised.* Elevated production of $\bullet\text{O}_2^-$ may also be linked to plaque instability (Cai and Harrison 2000; De Meyer et al 2003) with the shoulder region of the plaque being a particularly active area for $\bullet\text{O}_2^-$ production (Sorescu et al 2002). Patients with endothelial dysfunction and in whom arterial $\bullet\text{O}_2^-$ production is also elevated are at highest risk for vascular morbidity and mortality (Guzik et al 2000; Heitzer et al 2001; for review see Channon and Guzik 2002). In diabetes, where cardiovascular disease is of particular concern, there are multiple sources of ROS including the auto-oxidation

of glucose, increased substrate flux, and decreased levels of NADPH through the polyol pathway. Formation of advanced glycation end (AGE) products and their interaction with cellular targets, such as endothelial cells, may lead to oxidative stress and promote formation of oxidized LDL.

Several enzyme systems are known to be sources of ROS including the mitochondrial respiratory chain, xanthine oxidase, NADPH oxidase, cyclooxygenase, cytochrome P450, and uncoupled eNOS (Ellis and Triggler 2003). Brownlee's group (Nishikawa, Edelstein, Brownlee 2000; Nishikawa, Edelstein, Du, et al 2000) have argued that mitochondria are the source of ROS and that uncoupling, for instance, of oxidative phosphorylation in endothelial cells under high glucose conditions, prevents the sequelae of hyperglycemia. The work described by Nishikawa and colleagues (2000) was performed using cell culture protocols but cell culture conditions per se may result in oxidative stress (Halliwell 2003). Therefore, these data need to be reproduced in functional vascular preparations before conclusions can begin to be translated to clinical conditions. There is also growing evidence that NADPH oxidase is a major source of vascular superoxide production (Griendling et al 2000; Jiang et al 2004). For example, increased activity of NADPH oxidase makes an important contribution to the pathogenesis of experimental models of vascular disease, including intimal hyperplasia induced by periarterial collars (Paravicini et al 2002) and arterial balloon injury (Souza et al 2000; Chen et al 2004), cholesterol-induced atherosclerosis (Warnholtz et al 1999), vein graft intimal hyperplasia (West et al 2001), and hypertension (Zalba et al 2000). Gene disruption of the p47phox component of NADPH oxidase has been shown to significantly reduce superoxide production by vascular smooth muscle cells and, importantly, to reduce the development of atherosclerotic lesions (Barry-Lane et al 2001). A crucial clinical link was shown by Guzig et al (2000), who found that increased superoxide generation by NADPH oxidase in vessels was strongly associated with risk factors for atherosclerosis and impaired endothelial NO function in patients with coronary artery disease. In addition, there is experimental evidence that shows unambiguously that blocking NADPH oxidase-mediated generation of superoxide leads to regression or amelioration of vascular disease. Pharmacological and gene targeting strategies for NADPH oxidase have been found to lower blood pressure (Touyz 2004) and regress or reduce vascular remodeling (Barry-Lane et al 2001; Chen et al 2004; Dusting et al 2004). Intuitively, increasing antioxidant intake should therefore prove beneficial for cardiovascular disease.

So why then are the data from epidemiological and clinical studies with antioxidants often confusing and contradictory?

The data from intervention studies in humans with antioxidants, notably vitamin C and/or vitamin E (tocopherols), may simply reflect that the interventions produced variable reductions in oxidative stress in a highly heterogeneous population (see Antoniadou et al 2003). Many epidemiological and observational studies have provided support for the concept that a diet rich in antioxidants, despite exposure to other cardiovascular risk factors such as dietary fat, is associated with lower incidences of cardiovascular events (Gey et al 1991). Similarly for animal studies – although the data for vitamin E supplementation is open to other interpretations (Upston et al 1999). To take such data and design an appropriate prospective study is not an easy task. Unfortunately, several large prospective randomized intervention studies have failed to provide support for the benefit of antioxidants. Thus, the HOPE study with vitamin E (Lonn et al 2002; Mann et al 2004) and another study with vitamin C, vitamin E, and β -carotene (Heart Protection Study Collaborative Group 2002) reported no benefit for patients with diabetes and cardiovascular disease. In both these studies, plasma concentrations of the vitamins as well as the lipid profile were measured and reported, but surprisingly, no measure was made of oxidative stress. Thus, in neither study was it possible to conclude whether the interventions actually modified oxidative stress in the patients; somewhat akin, as noted by Halliwell (2000), to conducting a trial with antihypertensive drugs but not monitoring blood pressure. Although it can be argued that there is no clear agreement as to which biomarkers best monitor oxidative stress, the measurement of, for instance, the isoprostanes as an indicator of lipid peroxidation would have provided one reference set of data (see Griendling and FitzGerald 2003; Halliwell and Whiteman 2004). In addition, the choice of vitamins C and E may not have been the best antioxidants to include in the trials (they do not greatly affect isoprostane levels) (Levine et al 2001; Meagher et al 2001), although their inclusion was probably based on their ready availability in dietary sources. Vitamin E is associated with the lipophilic/hydrophobic domains of lipoproteins and cell membranes, and ROS are generated in the cytosolic and extracellular compartments – perhaps they never meet (Touyz 2004)? Furthermore, it is doubtful whether any of the classical antioxidants are capable of preventing the reaction of superoxide with NO, for this is one of the fastest known “biological” reactions (rate constant

$6.7 \times 10^9 \text{ mol}^{-1} \cdot \text{s}^{-1}$; Huie et al 1993), and is much faster than the dismutation of superoxide by phenolics such as vitamin E. Finally, the choice of the patient population selected for these trials may be questioned. If you are going to study the effects of antioxidant therapy would it not be best to choose a population group with demonstrated high levels of oxidative stress?

If vitamin C and E are not the ideal antioxidants, then what should be used?

Much recent press has been given to ubiquinone, or coenzyme Q₁₀, which has been described as a “powerful antioxidant” and, because it is a critical intermediate of the mitochondrial electron transport chain, can be readily linked to a mitochondrial dysfunction and elevated NADH and NADPH oxidase activity (see Chew and Watts 2004). Indeed oral coenzyme Q₁₀ improves brachial artery endothelial function in patients with dyslipidemia and type 2 diabetes (Watts et al 2002). These data are potentially of significance as the basis for intervention strategies in so far as they have been interpreted as reflecting a “targeted correction” of the cellular basis of oxidative stress. Is this a correct conclusion? The answer is “probably not”, as more studies are clearly required to directly demonstrate the link between coenzyme Q₁₀ treatment, improvement of endothelial function, and an action on the mitochondrial electron transport chain. Probucol, another so-called antioxidant that has proved moderately successful in preventing restenosis after coronary angioplasty (Cote et al 1999; Tardif et al 2003), also lowers cholesterol and induces a protective hemoxygenase enzyme (HO-1) in the artery wall (Deng et al 2004). Its benefits, therefore, cannot be ascribed entirely to its antioxidant properties.

Unfortunately the term “antioxidant” is widely misused, for just about any molecule can act as an antioxidant provided it is presented with an appropriate oxidizable substrate. Indeed, most antioxidants can become pro-oxidants under certain cellular circumstances – this is true for α -tocopherol (the most active form of vitamin E), which can be pro-oxidant and initiate tocopherol-mediated peroxidation (Bowry et al 1992; Upston et al 1999). Electron transfer to antioxidants can generate other free radicals that can have their own pathophysiological actions. Moreover, as discussed above, the reaction of NO with superoxide is much faster than the electron transfer between superoxide and classical antioxidants, so it is not surprising that when superoxide and NO are present in the same cellular

compartment, antioxidants have little impact on the pathways of oxidation.

So where do we go from here?

Potentially, blocking the source of ROS generation in pathophysiological circumstances may be a more fruitful approach to relieving oxidative stress and its consequences than attempting to inactivate superoxide after it is formed. Although there are several experimental tools that can be used to block NADPH oxidase in vitro or even in vivo (Brosnan 2004; Jiang et al 2004), none of the compounds in the public domain can be considered sufficiently selective to embark on development for clinical trial at this stage. For anything more than acute intervention, a compound that inactivates the NADPH oxidase system entirely could be expected to compromise infection control, as exemplified in chronic granulomatous disease, a genetic disorder resulting from a defect in the key catalytic subunit of the NADPH oxidase in leukocytes (Babior 2004). However, the discovery that there are distinct isoforms of this crucial membrane subunit expressed particularly in vascular cells including endothelium (Cai et al 2003; Jiang et al 2004; Ellmark et al 2004) opens the way to develop vascular-specific inhibitors of the NADPH oxidase. While such deliberately targeted compounds will become available in the near future, it is attractive to speculate upon the reasons why the most successful cardiovascular drugs of recent times (ACE inhibitors, angiotensin AT1 receptor blockers, and HMGCoA reductase inhibitors) have all been proven to greatly improve morbidity and mortality outcomes in large multicentre trials. All of these drugs indirectly reduce the activity of NADPH oxidase (Jiang et al 2004), because angiotensin II, acting through AT1 receptors, is a well known activator of the vascular NADPH oxidase (Cai et al 2003), and statins also block activation of the enzyme complex by the Rac subunit (Wagner et al 2000). Perhaps fortuitously, these powerful therapeutics are treating the underlying cause of vascular disease – oxidative stress. In the meantime, there is scope for a fuller elucidation of the pathways that lead to oxidative stress and the description and pharmacological characterization of powerful antioxidants that can be used in improved clinical trials. We can then answer the question: Are we over oxidized?

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