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FREE RADICALS IN CARDIOVASCULAR DISEASE

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FREE RADICALS

Free radicals damage compounds of all biochemical classes: nucleic acids, proteins, free amino acids, lipids, lipoproteins and connective tissue macromolecules. A significant part of the oxidative stress in biological systems is due to the production of oxygen-derived free radicals such as the hydroxyl, superoxide and peroxynitrite radicals, and related reactive oxygen species such as hydrogen peroxide and nitric oxide (Table 1).

TABLE 1
Free radicals and reactive oxygen species - chemical composition.

Molecular oxygen	O_2	
Superoxide anion	$O_2^{\bullet-}$	*Super oxide dismutase* (SOD) catalyses the breakdown of $O_2^{\bullet-}$ to hydrogen peroxide and oxygen.
Hydrogen peroxide	H_2O_2	Similar permeability to water. Generates hydroxyl radical in the presence of iron. Broken down to water and oxygen by *catalase*.
Hydoxyl radical	$OH^{\bullet}$	Generated from hydrogen peroxide or peroxynitrous acid. Very powerful oxidising agent.
Nitric oxide	NO	Formed by *nitric oxide synthase* (NO synthase). Reacts with superoxide to form peroxynitrite.
Peroxynitrite	$NO_3^{\bullet-}$	Generated from nitric oxide and the hydroxyl radical. Forms an acid (peroxynitrous acid) which degenerates to hydroxyl radical and nitrogen dioxide.

The term 'free radical' is used to describe any chemical compound that has an unpaired electron in its structure. This arrangement is highly unstable and thus free radicals have life-spans in the order of 10^{-8}-10^{-10} seconds, though some like nitric oxide persist longer, perhaps 1-2 seconds. Free radicals may be generated by endogenous reactions or exogenous agents (Table 2).

The unpaired electron within the structure of a free radical is capable of a vast array of chemical interactions. These are superbly covered by Halliwell and Gutteridge[1] and are beyond the scope of this article. However, free radicals are potent oxidising agents and one free radical can interact with another chemical component to produce separate free radicals. For example, the combination of superoxide and nitric oxide generates the peroxynitrite radical.

Free radicals have been implicated in several important biological processes: ageing, anti-microbial defence, mutagenesis and, more recently, in cardiovascular disease.[2,3] They have also been implicated in the pathogenesis of reperfusion injury,[3-7] endothelial damage[8] and atherogenesis.[7,9]

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TABLE 2
Sources of free radicals.

Endogenous *Mechanism*	*Examples*
Oxidation/reduction reactions	mitochondrial transport chain, xanthine oxidase, cyclo-oxygenase, NO synthase
Peroxidase activity	neutrophils, monocytes, macrophages, vascular endothelial cells.
Auto-oxidation reactions	iron, adrenaline, polyunsaturated fatty acids
Fenton Reaction	iron (normally protected by its incorporation within haemoglobin, myoglobin and transferrin).
Exogenous *Mechanism*	*Examples*
Redox-cycling drugs/chemicals	paracetamol, carbon tetrachloride, paraquat.
Drug oxidations	doxirubicin
Cigarette smoke	
Ionising radiation	

DEFENCE MECHANISMS

Extensive repair mechanisms to counteract free radical damage have evolved in biological tissues and these range from basic turnover of protein and lipid membranes to highly specific, highly efficient repair processes for RNA and DNA. In addition there are many agents which inhibit the generation, propagation or activity of free radicals (Table 3).

TABLE 3
Agents protective against free radical activity.

Protective agent	*Information*
Catalase	Large, water-soluble enzyme, abundant in red blood cells. Rapidly converts hydrogen peroxide to oxygen and water.
Superoxide dismutase	Large water-soluble agent which scavenges the superoxide anion, generating hydrogen peroxide. Exists in close proximity to catalase.
Haemoglobin/myoglobin	Dual role: can convert hydrogen peroxide to oxygen and water, but can also release free iron.
Glutathione	Reduced form maintained by NADPH and is gradually depleted under oxidative stress.
Ascorbate	General mild water-soluble reducing agent which may have an important role maintaining the integrity of haem.
∝-Tocopherol	Lipid soluble, works as a chain-breaking agent.
Ubiquinone	Significant contribution to the antioxidant effects of diet.
ß-carotene	Lipid soluble agent.

In vivo, and in health, there is a balance between free radical generation and the activity of preventive antioxidants, which are molecules in close proximity to the site of generation of the radical and which quench the reaction process before a radical generating cascade begins. These preventative antioxidants include catalase, superoxide dismutase (SOD) and haemoglobin. Other molecules act as chain-terminating agents, preventing the continuation of the free radical cascade and include agents such as vitamins E and C, and glutathione.

There appears to be a circadian rhythm in the production of free radicals and of the defence mechanisms.[10] This cycle may be disturbed in ischaemic heart disease.[11]

Currently research interest has focussed on trying to target effective therapeutic strategies to maintain the balance between free radical generation and antioxidant defence mechanisms by either preventing free radical generation or by supplementing the effectiveness of existing preventive and chain breaking antioxidants.

The micro-environment surrounding the vascular endothelium is a site where such oxidant/antioxidant conflict prevails. The vascular endothelium is particularly prone to oxidative damage for any one of four following causes: increased production of free radicals as by-products of local metabolic processes such as from polymorphonuclear neutrophil leucocytes (hydrogen peroxide) or xanthine oxidase (superoxide); cigarette smoke; high levels of oxidised LDL cholesterol; and diabetes mellitus. There is evidence to suggest the importance of the vascular endothelium as the principal site of action for cardiovascular risk factors in early atherogenesis.[12]

ENDOGENOUS ENZYMATIC FREE RADICAL GENERATING SYSTEMS

Xanthine oxidase

Xanthine oxidase (XO) is the catalyst for the metabolic degradation of hypoxanthine to uric acid, and therefore plays an important role in purine metabolism. As a by-product of this reaction XO produces predominantly superoxide, but also hydrogen peroxide and the hydroxyl radical.[13] There has been increasing interest in xanthine oxidase (XO) as a free radical enzyme-generating system, particularly because allopurinol, a competitive antagonist of XO, is cheap and effective and, therefore, of potential therapeutic benefit as an antioxidant.

Work *in vitro* in isolated tissues[14] and in animals[15] has shown that XO may be a major source of free radical generation. Further animal and human models have shown that treatment with an XO antagonist may be of benefit in reducing oxidative stress during cardiovascular surgery.[16,17] Allopurinol has been given to patients during coronary artery bypass grafting, and found to improve a variety of haemodynamic and biochemical parameters.[18-21] There is also one report of a reduction in mortality,[22] although this has not been consistently confirmed by other studies.[23]

Nitric oxide

Nitric oxide (NO) is produced widely in the body by the enzyme NO synthase. NO acts as a neurotransmitter both centrally and peripherally,[24,25] plays a crucial role in platelet aggregation and also in the regulation of cardiac contractility.[26] NO is also important in immunological defence[27,28] being produced by both neutrophils[8] and macrophages.[29]

Endothelial dysfunction is known to precede atherosclerosis.[12] A marker of endothelial dysfunction is nitric oxide production and this synthetic process is abnormal in essential hypertension,[30] smoking[31] and non-insulin dependent diabetes mellitus.[32] Treatment with L-arginine, a precursor of endothelium-derived NO, can restore endothelial dysfunction in hypercholesterolaemic patients.[33]

Studies suggest that NO is not always a beneficial autocoid; it readily forms the peroxynitrite and hydroxyl radicals in the presence of oxygen and other free radicals.[34] The clinical consequences can be profound, for example, in septic shock NO is generated in abundance by neutrophils and macrophages activated by endotoxin, and the administration of NO synthase inhibitors can reverse a proportion of the hypotension associated with this form of shock.[35]

A potential for harm exists if NO binds inappropriately to other transition metal compounds (haem or iron-sulphur centres for other enzymes).[36] In excess, NO can also inhibit mitochondrial respiration,[37] damage DNA[38] and reduce the replication of vascular smooth muscle cells.[39]

SMOKING

Cigarette smoking continues to be a major public health issue, made even more pertinent by the escalating numbers of young adults who smoke.[40] This highlights two major facts: firstly, the failure of public health education targeted at young adults (or perhaps the converse success of advertising); and secondly, the potential future burden of pathology due to smoking on health care resources, as this cohort become the patients of the future.

Smokers have a two and a half fold increase in risk of developing coronary artery disease compared with non-smokers[41] and there is a linear relationship between the dose of exposure to cigarette smoke and the severity of atherosclerotic disease in both animals[42] and man.[43] Over 4,000 chemicals are contained in tobacco smoke,[44] some of which may increase free radical stress,[45] which in part may be responsible for the observed endothelial dysfunction and decreased bioavailability of NO.

Evidence that the superoxide free radical may be a significant culprit has emerged recently.[46] Biochemical markers of free radical activity, in particular lipid peroxides, are elevated in smokers,[45,47] and levels of malondialdehyde (a marker of lipid peroxidation) are increased in normal volunteers who smoke.[48] Treatment with the antioxidants, vitamin C and E can reduce the level of free radical altered derivatives of arachadonic acid, such as 8-*epi*-prostoglandin-$F_{2\alpha}$, in chronic smokers.[45] Smokers tend to have a lower dietary intake of cereals and their derivatives (a major source of vitamin E) and consequently tend to have lower plasma levels of a tocopherol,[49] esterified fatty acids,[50] and other dietary antioxidants.[51]

HYPERCHOLESTEROLAEMIA

A defect of NO function is detectable in hypercholesterolaemic patients.[52] It is unclear whether the defect is one of diminished production or exaggerated breakdown of NO. Some evidence would suggest the latter, as concentration of some breakdown products of NO (nitrite and nitrate) are elevated in hypercholesterolaemia;[53] rather its activity may be impaired due to degradation by enhanced free radical activity.[52] Consequently one possible way in which the improvement in endothelium-dependent vasodilation (mediated by locally produced NO), which is associated with lowering of cholesterol concentration may occur, is by a reduction in the production of oxygen-derived free radicals.[52] This in turn could change the oxidative metabolism of NO allowing it to act as a vasodilator. A reduction in serum cholesterol produces normalisation of oxygen-derived free radical production and a combination of cholesterol-lowering therapy with and without antioxidant therapy, has been shown to have a beneficial effect on coronary vasomotion.[54,55] The picture is still unclear, however, as a study by Garcia *et al* found that endothelial dysfunction was not associated with increased extracellular destruction of NO by superoxide anions.[56]

It appears that the vascular impairment seen with hypercholesterolaemia is particularly associated with a subfraction of LDL which has been oxidised, termed ox-LDL. Galle *et al*[57] demonstrated that the NO released by endothelial cells was removed by ox-LDL. Schmidt *et al*[58] have demonstrated similar findings and that there was a dose-

dependent relationship between NO consumption and ox-LDL levels. If the oxidised components of ox-LDL, such as lysophospholipids and lipid peroxides, are neutralised, then the degree of endothelial dysfunction is also attenuated.[59]

Cholesterol-mediated endothelial dysfunction can occur in children[60] and surprisingly, differences are demonstrable after a single fatty meal in adults.[61] Possible sources of superoxide free radicals are endothelial cells, migrating monocytes or macrophages.[62]

Improved endothelial function may in part also assist to explain the evidence that lipid reduction reduces macrovascular events, including fatal cardiovascular deaths, both in primary[63] and secondary prevention studies.[64,65] How much of the benefit is due to reduced oxidative stress remains to be determined.

DIABETES MELLITUS

Oxidative stress increase plays an important role in the of diabetic complications.[2,66,71] The generation of free radicals may be increased in patients with diabetes through several biochemical pathways such as glucose autoxidation, polyol pathway activation, prostanoid synthesis through the cyclo-oxygenase pathway and protein glycation; antioxidant reserve may be reduced.[67,68] Free radicals also accelerate the formation of advanced glycosylation end-products (AGEs) which in turn can generate more free radicals.

LDL cholesterol is both oxidised and glycated in diabetes mellitus.[69] Ox-LDL has been shown to have a significant impact on the NO pathway in diabetic patients resulting in decreased endothelium-dependent vasodilation and increased synthesis of endothelin.[70] Both oxidised and glycated LDL induce the transformation of macrophages into foam cells.[71]

Antioxidant status: Acute elevations in glucose may depress the levels of natural antioxidant defences.[68,70] Antioxidant status is altered in young people with insulin dependent diabetes mellitus (IDDM).[72] In general, antioxidant enzymes such as SOD are raised in IDDM, although reduced or unchanged levels have also been reported.[70] The initial response to free radical activity could be raised SOD levels followed by depletion when the enzyme becomes overwhelmed. In erythrocytes from diabetic patients, levels of malondialdehyde are raised and levels of glutathione, an antioxidant, are decreased.[73]

Vitamins E and C are the major non-enzymatic antioxidants and their levels may be decreased in diabetes, although this is not a consistent finding.[68,70] There is very little information regarding antioxidant status in blood vessels themselves and, therefore, the direct effect on endothelial cell dysfunction remains unclear.

ANTIOXIDANT THERAPY

The imbalance between pro-oxidants (free radicals and reactive oxygen species - Table 2) and antioxidants in the development of atherosclerosis has prompted the investigation of antioxidant supplementation as possible therapy. Data from epidemiological studies suggests that a low dietary intake of antioxidants, in particular vitamin E, is associated with a higher risk of vascular disease.[74] A relatively low dietary intake of antioxidant vitamins, especially in smokers, is most likely to contribute to the higher incidence of coronary heart disease (CHD) in Scotland and Northern England.[75] In comparison, the incidence of CHD is lower in Mediterranean countries where the consumption of vitamin E, mostly from olive and cereal oils, and of vitamin C from citrus fruits is high.[76]

Observational studies show that the use of vitamin E supplements is associated with a reduced incidence of CHD.[77,78] The results from these studies must, however, be viewed with caution because the subjects were not necessarily representative of the normal population. Neither of the two controlled trials of antioxidant therapy showed any cardiovascular benefit.[79,80] The recent Cambridge Heart Antioxidant Study (CHAOS) data suggested that myocardial infarction, though not mortality, could be reduced using vitamin E supplementation.[81]

The evidence for a protective effect of vitamin C is weaker although deficiencies have been reported in patients with peripheral vascular disease compared with normal control subjects.[82] In patients with diabetes mellitus, vitamin C supplementation inhibits early protein glycation.[83] Additionally, Ting *et al* [84] showed that acute infusions of vitamin C augmented endothelium-dependent blood flow in patients with type II diabetes.

In animal model of diabetes, the antioxidant a-lipoic acid improves neural blood flow by reducing oxidative stress.[85] More recently in patients with non-insulin dependent diabetes mellitus (NIDDM), oral treatment with α-lipoic acid has been shown to improve cardiac autonomic neuropathy[86] by improving neural blood flow and further use of this compound is under investigation.

Clearly, more randomised trials are required to determine the true effects of antioxidant supplementation. In particular, trials of combinations of more than one antioxidant are required. In the meantime, it is advisable to eat foods high in vitamins E and C, such as nuts, olive oil, cereals, and fresh fruit and vegetables.

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