What Is Oxidative Stress?

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Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, is discussed in relation to its possible role in the production of tissue damage in diabetes mellitus. Important free radicals are described and biological sources of origin discussed, together with the major antioxidant defense mechanisms. Examples of the possible consequences of free radical damage are provided with special emphasis on lipid peroxidation. Finally, the question of whether oxidative stress is increased in diabetes mellitus is discussed.

THE PURPOSE OF THIS short review is to provide the reader with a simple introduction to the concept of oxidative stress as a potentially important contributor to tissue damage. The role of oxidative stress has been hypothesized in many clinical situations, but here attention will be focused on its possible role in the microvascular and macrovascular complications of diabetes.

Oxidative stress will be defined along with a description of the origin of reactive oxygen species and other free radicals. Because of the potential for catastrophic damage as a result of free radical attack, complex antioxidant defense mechanisms have evolved to protect body tissues. These processes will be discussed together with reference to their possible deficiencies in the diabetic state. Examples will be provided of the potential for free radical attack on important cellular components, with particular emphasis on lipid peroxidation.

Generally, the chemical reactions involved in free radical damage occur almost instantaneously so that direct measurement is a major problem. Some of the various methods that have been developed to assess oxidative stress that concentrate mainly on the detection of the consequences of free radical attack will be described. Finally, the potential role for oxidative stress in diabetes will be introduced to provide a background to the reviews that follow. The close interaction between glycation and oxidative stress is likely to be important in the etiology of diabetic tissue damage. The reader is referred to recent reviews for more detailed descriptions of the biochemical processes involved in oxidative stress.[1-6]

WHAT IS OXIDATIVE STRESS?

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, which may lead to tissue injury.[1]

Free radicals are formed in large amounts as an unavoidable by-product of many biochemical processes and in some instances, deliberately, such as in activated neutrophils. In addition, free radicals may be generated in the body in response to electromagnetic radiation from the environment and acquired directly as oxidizing pollutants such as ozone and nitrogen dioxide. If antioxidant defenses are deficient then damage may occur in a variety of tissues.

WHAT ARE FREE RADICALS?

Free radicals can be defined as any chemical species that contains unpaired electrons. Unpaired electrons increase the chemical reactivity of an atom or molecule. Common examples of free radicals include the hydroxyl radical ($^{\cdot}OH$), superoxide anion ($O_2^{\cdot -}$), transition metals such as iron and copper, nitric oxide ($NO^{\cdot}$), and peroxynitrite ($ONOO^-$).

The hydroxyl radical, the most potent oxidant known, has an extremely short half-life, reacting at the site of its formation through its ability to attack most biological molecules resulting in the propagation of free radical chain reactions. Superoxide is formed when oxygen accepts an electron and is not in itself particularly reactive. It can act as a weak oxidizing agent, but is much stronger as a reducing agent of iron complexes such as cytochrome C. It is likely to be more important as a source of hydroxyl radicals and hydrogen peroxide. Nitric oxide, an example of a physiological radical, is of considerable interest through its role as a mediator of vascular tone.[7]

WHERE DO FREE RADICALS COME FROM?

Free radicals can be produced by several different biochemical processes within the body including: reduction of molecular oxygen during aerobic respiration yielding superoxide and hydroxyl radicals; by-products of chemistry such as oxidation of catecholamines and activation of the arachidonic acid cascade product electrons, which can reduce molecular oxygen to superoxide; production of superoxide and hypochlorous acid (HOCl), a powerful oxidant, by activated phagocytes; nitric oxide production by vascular endothelium and other cells. In addition, free radicals can be produced in response to external electromagnetic radiation, such as gamma rays, which can split water to produce hydroxyl radicals (Fig 1).

THE POSSIBLE CONSEQUENCES OF FREE RADICAL-MEDIATED TISSUE DAMAGE

Free radicals are inherently unstable molecules because of the presence of unpaired electrons. As a result, they can be highly reactive, although this varies from radical to radical, reacting locally to accept or donate electrons to other molecules to achieve a more stable state. As most molecules are not free radicals, the majority of reactions will involve nonradicals. Reaction of a radical with a nonradical (all biological macromolecules, lipids, proteins, nucleic acids, and carbohydrates are possible targets) produces a free radical chain reaction with the formation of new radicals, which in turn can react with further

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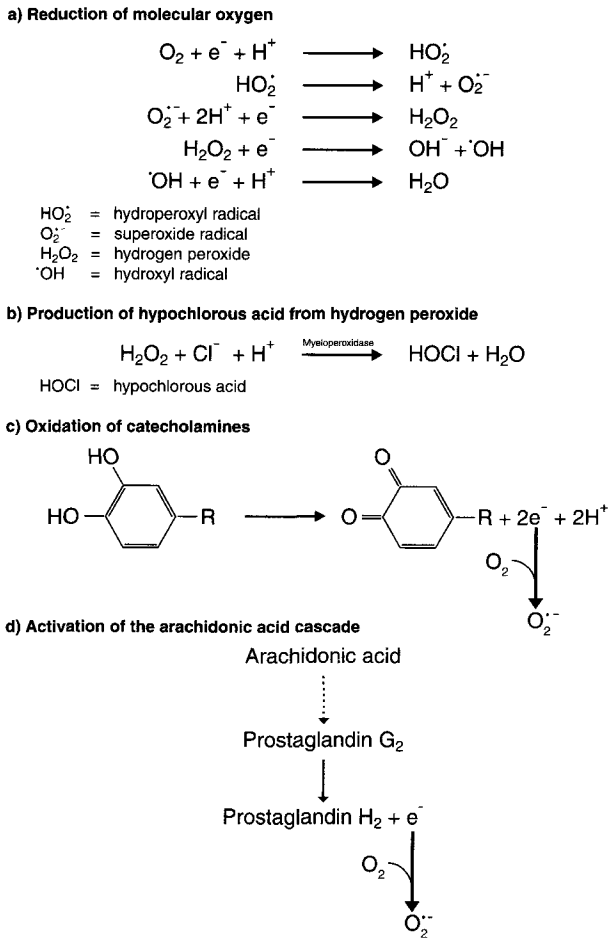

Fig 1. Examples of the production of free radicals by different biochemical processes within the body.

macromolecules. Important examples are lipid peroxidation and protein damage, eg, addition of carbonyl groups or crosslinking or fragmentation. Carbonyl derivatives of amino acid residues render the protein susceptible to proteolysis,[8] DNA base (eg, conversion of guanine into 8-hydroxyguanine and other products) single- and double-strand breaks, and protein/DNA crosslinks.[9-11] Lipid peroxidation is perhaps the most extensively studied consequence of free radical attack and is of potential importance in diabetic vascular damage. For these reasons, lipid peroxidation will be described in some detail (Fig 2).

Reactive free radicals, eg, the hydroxyl radical, have the capacity to abstract a hydrogen atom ($H^\cdot$) from a methylene group (—CH_2—) of fatty acids, leaving behind an unpaired electron on the carbon (—$\dot{C}H$—). Polyunsaturated fatty acids are particularly prone to free radical attack because the presence of a double bond weakens the carbon-hydrogen bond at the adjacent carbon atom. The remaining carbon-centered radical undergoes molecular rearrangement resulting in a conjugated diene. Conjugated dienes can combine with oxygen forming a peroxyl radical. This is itself able to abstract a further hydrogen atom and begin a chain reaction that continues either until the substrate is consumed or the reaction is terminated by a chain-breaking antioxidant such as vitamin E. The resulting lipid peroxides are reasonably stable compounds, but their decomposition can be catalyzed by transition metals and metal complexes producing alkoxyl and peroxyl radicals, which can stimulate further lipid peroxidation. It is of interest that disrupted tissue is more susceptible to lipid peroxidation. Lipid peroxidation can have profound effects on cellular function. Extensive peroxidation in cell membranes will result in changes in fluidity, increased permeability, a decrease in membrane potential, and eventually membrane rupture.

Over recent years, it has become clear that oxidation of low-density lipoprotein (LDL) is central to many of the processes of atherogenesis.[12,13] LDL cholesterol explains the strong, positive, independent relationship between total plasma cholesterol and atherosclerosis-related disease particularly coronary heart disease. Steinberg et al[14] have hypothesized that oxidatively-modified LDL is the atherogenic particle. Important cells in the atherogenic process, arterial endothelial cells, smooth muscle cells, and macrophages are all able to oxidize native LDL. Oxidized LDL can be taken up by monocyte/macrophages through a scavenger receptor. This receptor uptake mechanism, unlike the classical LDL receptor, is not downregulated by increased cellular cholesterol ester content such that lipid-laden foam cells are formed. Accumulation of these cells (the fatty streak) in the subendothelial space

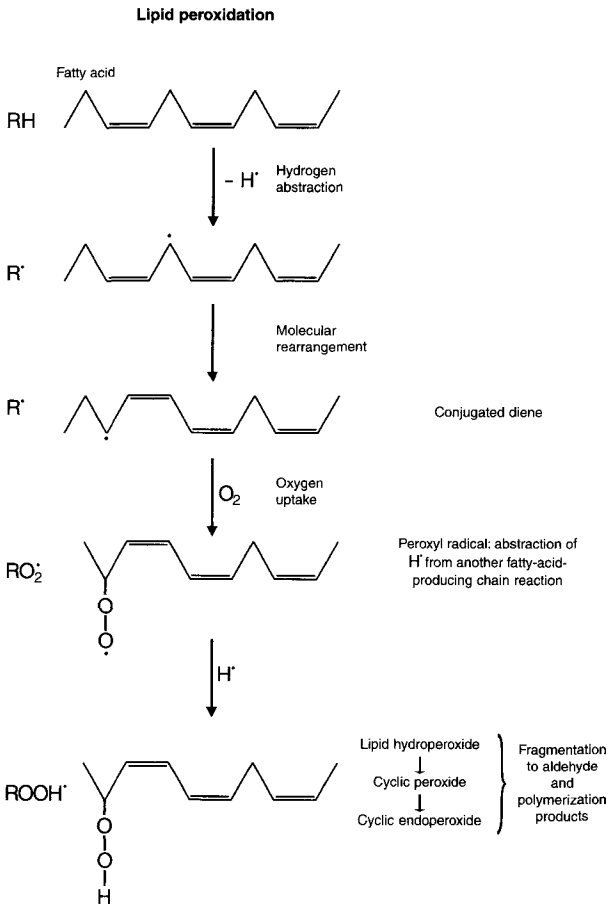

Fig 2. Lipid peroxidation as an example of free radical-mediated tissue damage. Reprinted by Gutteridge.[3]

damages the overlying endothelium, which allows platelets to aggregate and release powerful mitogens that contribute to the further development of the lesion.

LDL oxidation results in extensive fragmentation of LDL polyunsaturated fatty acids with conjugation of the fragments to LDL apoprotein B and phospholipid. Lecithin is converted to lysolecithin by LDL-associated phospholipase A_2. At a later stage, there is fragmentation of LDL apoprotein B forming a new configuration, which is recognized by the scavenger receptor. Oxidatively-modified LDL also has additional properties that render it more atherogenic than native LDL including: direct cytotoxicity, induction of adhesion and chemoattractant molecules, inhibition of endothelium-dependent relaxation factor and increased expression of tissue factor, activation of platelets and T cells, stimulation of smooth muscle cell growth, stimulation of plasminogen activator inhibitor-1 and endothelin from endothelial cells, and immunogenicity.[15]

WHAT ARE THE TISSUE DEFENSES AGAINST FREE RADICAL ATTACK?

Given the potential for tissue damage, it is perhaps not surprising that the body has evolved major antioxidant defense mechanisms to protect it from free radical attack. These defenses can be conveniently considered as cellular, membrane, and extracellular mechanisms. Gutteridge[3] has defined an antioxidant as any substance that, when present at low concentrations, compared with those of the oxidizable substrate, considerably delays or inhibits oxidation of the substrate. Cellular antioxidant defenses include the dismutase, peroxidase, and catalase enzymes. In addition, the potential for intracellular free radical production is greatly diminished by the ability of mitochondrial cytochrome oxidase to function catalytically in the electron transport chain without releasing reactive oxygen species.[16] Superoxide dismutases (containing copper and zinc at the native site) in cytosol and in mitochondria (containing manganese) catalyze the dismutation of superoxide to hydrogen peroxide and oxygen:

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

The product of this reaction, hydrogen peroxide, is a weak oxidant and is relatively stable. However, unlike superoxide, hydrogen peroxide can rapidly diffuse across cell membranes, and in the presence of transition metal ions it can be converted to hydroxyl radicals via Fenton chemistry:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$

Two enzyme systems can break down hydrogen peroxide. Glutathione peroxidases present in cytosol and mitochondria have a major role in removing hydrogen peroxide generated by superoxide dismutase with the oxidation of glutathione (GSH):

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

Catalases that are present in peroxisomes in many tissues remove hydrogen peroxide when present in high concentrations:

$$2H_2O_2 \rightarrow O_2 + 2H_2O$$

Antioxidants such as vitamin E, β-carotene, and coenzyme Q are present within cell membranes. The lipophilic vitamin E (α-tocopherol) is a highly effective antioxidant when incorporated in the lipid core of cell membranes. It has the ability to scavenge intermediate peroxyl radicals and, therefore, interrupt the chain reaction of lipid peroxidation. For this reason, vitamin E is described as a chain-breaking antioxidant.[17]

$$\begin{array}{ccc} O_2^{\bullet} & & O_2H \\ | & & | \\ \text{-C-} + TH \rightarrow & & \text{-C-} + T^{\bullet} \end{array}$$

(α-tocopherol)

The tocopherol radical ($T^{\bullet}$) is much less reactive and is converted back to α-tocopherol by vitamin C. In addition to the presence of antioxidants, membrane structure with appropriate proportions of cholesterol and phospholipids is important in resistance to free radical attack.

Major extracellular antioxidant defenses include the metal-binding proteins. As discussed earlier, the free metals iron and copper can promote free radical damage, accelerating lipid peroxidation and catalyzing hydroxyl radical formation. The body is protected against these potentially adverse effects by binding proteins (transferrin, lactoferrin, and ceruloplasmin), which ensure that these metals are maintained in a nonreactive state. Similarly, haptoglobins, hemopexin, and albumin bind hemoglobin and heme, which can also accelerate lipid peroxidation. In addition to the major protective role of the metal-binding proteins, various low–molecular-weight molecules such as bilirubin, vitamin C, and urate have antioxidant properties.[18,19] Furthermore, distinct, extracellular forms of glutathione peroxidases and superoxide dismutases have been described.[20,21]

HOW IS OXIDATIVE STRESS MEASURED?

As described earlier, it is currently not possible to measure reactive oxygen species directly so attempts at the evaluation of free radical production and the pro-oxidant/antioxidant balance is based on markers of oxidative reactions often in in vitro experiments. It is important to bear in mind that differing experimental conditions, for example, the type of substrate employed and the nature of oxidative conditions applied, will result in the generation of different products.

In general terms, oxidative stress can be assessed by measurement of reaction products of oxidative damage, for example, lipid peroxidation, DNA oxidation, and protein oxidation. A complementary approach is the measurement of depletion of antioxidants such as α-tocopherol, vitamin C, and thiol groups. Lipid peroxidation is the most studied with a variety of techniques employed including thiobarbituric acid-reactive material assays, conjugated dienes, hydroperoxides, F_2 isoprostanes, and nitroxides.[22] Recently, Visioli and Galli[23] have provided an overview of the evaluation of oxidative processes in relation to cardiovascular disease.

Although oxidative stress has been implicated in cardiovascular disease and many other conditions, it is important to bear in mind, as Halliwell[1] has emphasized, that implicated does not mean important, and the relationship between oxidative stress and the onset and progression of disease processes is not fully

established. Furthermore, oxidative damage may be a consequence of a particular pathological process rather than its cause. Hopefully, with improved techniques such as electron spin resonance[24] discussed elsewhere in this volume, further insight into the role of oxidative stress will be obtained.

IS OXIDATIVE STRESS INCREASED IN DIABETES MELLITUS?

There is considerable evidence that oxidative stress is implicated in the development of diabetic complications.[25] Furthermore, the close interrelationships between the processes of glycation and advanced glycation end product (AGE) production, a major pathogenetic mechanism for diabetic complications,[26-28] and oxidative stress have been emphasized. The term "glycoxidation" has been coined to describe this concept.[29] It is beyond the scope of this article to describe these processes in detail and the reader is referred to the comprehensive reviews previously cited.

Several factors may contribute to increased potential for production of free radicals in diabetes including glucose auto-oxidation,[30] redox imbalance,[31] and AGE/receptor interactions.[32] In addition, free radical defense mechanisms may be reduced. As a consequence of glycation, proteins containing transition metals may undergo conformational change with the potential for increased availability of metal ions to catalyze.[33,34] In addition, Cu-Zn superoxide dismutase is inactivated by glycation,[35] and antioxidants (eg, vitamin E, vitamin C, catalase, and reduced glutathione) have been reported to be decreased in diabetes.[36,37]

The interaction of glycation and oxidant processes is well-illustrated by LDL modification, which is central to atherogenesis. Recently, the possible mechanisms by which LDL oxidation is promoted in the diabetic state have been reviewed.[38,39] Glycoxidation, the myeloperoxidase and reactive nitrogen pathways, and other mechanisms including the action of lipoxygenase together with reduced antioxidant mechanisms are all likely to contribute to increased LDL oxidation. Furthermore, the change in LDL subfraction distribution towards smaller, denser particles, which is well-described in type 2 diabetes,[40,41] increases susceptibility to oxidation.[42]

As discussed previously, the measurement of oxidative stress in vivo is difficult.[43] In our laboratory, this problem was approached using the ferrous oxidation with xylenol orange assay coupled with the selective hydroperoxide reductant triphenylphosphine to determine levels of lipid hydroperoxides in type 2 diabetic patients.[44-47] Plasma lipid hydroperoxides were significantly higher in the diabetics compared to controls. The ratio of lipid hydroperoxides to cholesterol standardized α-tocopherol was also significantly higher in diabetic patients. No differences were observed in lipid hydroperoxides or α-tocopherol between diabetics with or without clinical complications. A significant relationship was observed between the ratio of lipid hydroperoxide to cholesterol standardized α-tocopherol and fasting blood glucose in the diabetics but not with hemoglobin A_1, plasma cholesterol, or triglyceride. These data suggest that the elevated plasma hydroperoxides are associated with diabetes itself rather than the presence of complications, and perhaps that acute hyperglycemia is a more significant determinant than chronic glycemia as reflected by the hemoglobin A_1. Recently, plasma hydroperoxides measured by the techniques previously described have been shown to decrease with continuous subcutaneous insulin infusion compared to conventional insulin therapy in type 1 diabetic patients.[48] In this study, plasma hydroperoxides did correlate significantly with glycated hemoglobin levels during the study and with the level of microalbuminuria.

A further approach adopted in our own studies has been the measurement of products formed during peroxidation. The F_2-isoprostanes are a series of prostaglandin F_2-like compounds formed during arachidonic acid peroxidation independent of the cyclooxygenase pathway.[49] One of these compounds, 8-epi-$PGF_{2\alpha}$ has received considerable interest because of its role as a potent vasoactive mediator.[50] Patients with type 2 diabetes were found to have significantly higher plasma 8-epi-$PGF_{2\alpha}$ concentrations measured by gas chromatography-mass spectrometry/negative ion chemical ionization compared to controls.[51] In this study, no correlations were observed between concentrations of 8-epi-$PGF_{2\alpha}$ and glycated hemoglobin A_1, plasma glucose, plasma cholesterol, or triglyceride.

CONCLUSION

A concise, simplified introduction to the concept of oxidative stress has been provided in this short review together with its relevance to diabetes and diabetic complications. The close interaction between glycation and oxidative processes has been discussed. It should be apparent that this is a complex area and much is still to be learned. Indeed, although there is considerable evidence for increased oxidative stress in diabetes, this is not supported by some studies.[52,53] For instance, the level of advanced glycation end-products carboxymethyl lysine and pentosidine present on collagen in diabetes could be explained by mechanisms other than oxidative stress.[52] Furthermore, oxidized amino acids, methionine sulfoxide, and ortho-tyrosine in skin collagen were not increased in diabetes.[53] However, these observations certainly do not exclude more localized tissue-specific oxidative stress.

Hopefully this review will provide the basis for the subsequent articles in this volume that will explore further some of the important concepts raised, along with a discussion of the potential beneficial effects of the sulphonylurea gliclazide on aspects of oxidative stress.

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