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Editorial on Recent Advances

Oxygen toxicity: membrane damage by free radicals

The oxygen-rich atmosphere in which all obligate aerobes thrive has been an obvious advantage for our long-range biological evolution. Yet recent investigations indicate that oxygen may be a "two-edged sword," vital for our very existence on the one hand, but capable of insidious cellular destruction on the other. At the heart of this apparent paradox are a series of chemical reactions whereby oxygen is converted to a number of transient free radicals, highly reactive substances containing unpaired electrons. Free radicals are thought to be responsible for producing irreversible damage to biomolecules such as enzyme proteins and membrane lipids. These same free radicals may be causative agents of slower, ubiquitous aging processes.

Classical enzymology has established that during biological oxidations most cellular oxygen is reduced by two-electron transfer through the cytochrome carriers and other well-known redox systems. But recently, alternate pathways have been described whereby a small part of the cellular molecular oxygen can be reduced through univalent pathways, with the production of highly reactive free radicals.[1] The complete reduction of one molecule of oxygen requires four electrons. If this were to procede by univalent steps, several intermediates would be produced: superoxide anion radical ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). We now know that all oxygen-metabolizing cells have evolved protective mechanisms that either minimize the production of free radicals or, alternatively, destroy them as rapidly as they are formed.

One such mechanism involves superoxide dismutase (SOD), an enzyme that catalyzes, by dismutation, the following overall reaction[1]:

$$2\ O_2^{\cdot -} + 2\ H^+ \longrightarrow H_2O_2 + O_2$$

SOD is present in all respiring cells examined to date. Specific types are localized in either mitochondria or cytosol; they may contain copper and zinc on the one hand, or manganese or iron on the other. SOD comprises the major defense against accumulation of the highly reactive superoxide radical.

Granted the ubiquitous distribution of SOD and its importance in destroying superoxide anion radical, the above equation shows that in so doing, it produces hydrogen peroxide. Although hydrogen peroxide is a more stable and less reactive molecule, it is still highly toxic to the cell

since it can, by reaction with $O_2^{\cdot-}$ or with Fe^{+2} produce the extremely reactive hydroxyl free radical ($OH^{\cdot}$).[1, 2] As a second defense then, against a buildup of hydrogen peroxide, a battery of enzymes have evolved that reduce it to harmless water. These include catalase (found mainly in liver, kidney, and erythrocytes) and the peroxidases, a group of enzymes found in a variety of cell types (e.g., leukocytes, mammary, thyroid, and salivary glands, and most recently, in retinal pigment epithelium). Peroxidases acting on hydrogen peroxide require a co-substrate, or hydrogen donor. This may be a physiological reductant such as glutathione, ascorbic acid, or cytochrome c, or a synthetic dye such as *p*-phenylenediamine or diaminobenzidine. The physiological reductants are self-renewable by the reduced forms of nicotinamide adenine dinucleotide phosphate (NADPH) or nicotinamide adenine dinucleotide (NADH).

Many free radicals, regardless of their source, are capable of acting on biomembranes, and specifically on their polyunsaturated fatty acids (PUFA's).[2] This results in the formation of a lipid free radical ($L^{\cdot}$) which, in the presence of oxygen, is readily converted to lipid peroxide radical ($LO_2^{\cdot}$). The $LO_2^{\cdot}$ is a highly reactive species which triggers a chain propagation reaction by interacting with an adjacent PUFA molecule in the membrane. This process, known as autooxidation, is self-perpetuating in the presence of oxygen, and is thought to play the leading role in membrane damage. It is precisely at this intracelluar site that a third line of defense against oxygen toxicity exists, namely, free radical scavengers. Certain substances, notably α-tocopherol (vitamin E), are able to intercept or terminate the autooxidative chain reaction and thereby protect the membrane against further damage. The validity of this concept has been substantiated by recent work showing extensive degeneration of rod outer segment (ROS) membranes in vitamin E–deficient monkeys,[3] with a concomitant buildup of lipofuscin in the pigment epithelium. Vitamin E is an integral component of ROS membranes, and its depletion would be expected to adversely affect these polyunsaturated fatty acid–rich membranes, especially when challenged with oxygen.[4] The structural damage is due to peroxidation of ROS membrane fatty acids, the principal one being docosahexaenoate, a 22-carbon fatty acid containing six unsaturated double bonds (22:6). This species is highly susceptible to autooxidation by the mechanisms described above.

In addition to initiating a peroxidative chain reaction, the lipid peroxide free radical ($LO_2^{\cdot}$) can also decompose, forming highly reactive fragments such as malonaldehyde. This substance, a three carbon dialdehyde ($OHC\text{-}CH_2\text{-}CHO$), readily forms cross-linkages (through Schiff bases) with free amino groups of proteins, phospholipids, and nucleic acids, giving rise to high molecular weight fluorescent polymers, and at the same time immobilizing functionally important enzymes in cellular and subcellular membranes. These chemically damaged cell organelles appear to be autophagocytized by the cell's lysosomal system, but owing to their relative insolubility and indigestibility (or due to lack of appropriate enzymes), they cannot be completely degraded. These indigestible biomolecules accumulate within the lysosomal compartment of the cytoplasm and are recognized structurally as late-stage secondary lysosomes or residual bodies. Classically, they are known as lipofuscin or age pigments. They have characteristic fluorescence spectra,[2, 5] but are morphologically heterogeneous due to variations in the molecular structure of the original damaged organelles.

The lipid peroxide free radical may also be converted to hydroperoxide (LOOH) which, although less reactive than $LO_2^{\cdot}$, is nevertheless able to enter into the autooxidative chain reactions described above. To minimize this, another protective system exists in the cell which inactivates LOOH by converting it to a harmless hydroxy fatty acid (LOH). This reaction utilizes glutathione peroxidase, a seleno-

enzyme which has been detected in many ocular tissues. Selenium and vitamin E are thought to act synergistically in protecting cells from oxygen damage.

Where do these damaging free radicals originate in biological systems? They arise not only from the univalent reduction of oxygen, but also as by-products of ionizing radiation, ultraviolet light, and even visible light. Ocular tissues are prime targets for radiation-induced free radical damage, and during the past decade, the deleterious effects of light on the retina have indeed been firmly established by a number of investigators.[6, 7] Continuous cool illumination with even ordinary fluorescent lights leads to profound changes in photoreceptor function and morphology. The albino rat is especially susceptible to this kind of light damage. Noell and associates[6] postulated that "photosensitized oxidations" leading to the formation of lipid peroxides could play an important role in light damage, and direct evidence supporting this hypothesis was recently provided using the frog.[8] It was shown that lipid hydroperoxides were formed in both whole retina and ROS after only 30 minutes exposure to light in vivo. It is highly probable that the production of lipid hydroperoxides was mediated by light-induced free radical alteration of ROS membranes. There is also speculation that such changes in membrane structure could trigger the shedding of terminal ROS disks. Superoxide dismutase may enter the picture here since this enzyme has recently been demonstrated in retina and its highest activity is in ROS.[9]

Biochemical damage to the retinal pigment epithelium (RPE) by light and/or oxygen should be re-examined in view of the free radical character of melanin and its possible role as a biological electron-transfer agent.[10] Melanin appears to be unique among free radicals in its relative stability, yet it can efficiently transfer electrons from NADH to ferricyanide,[11] and also bind paramagnetic transition metal ions such as manganese, copper, or iron.[12] Recent evidence suggests that melanin can absorb excited state (i.e., free radical) energy and channel it into processes yielding highly cytotoxic substances.[13] Melanin is probably neither a source nor a sink for free radicals, but rather an efficient transfer agent for a variety of ionic species. Thus, although some of the free radicals in RPE cells may be absorbed and transformed by the melanin granules, others may be involved in the disposal of phagocytized ROS disks. It is tempting to speculate that a free radical–initiated autooxidation of the PUFA of ROS membranes takes place during or immediately after phagocytosis of shed ROS by the RPE. Both peroxidase and superoxide dismutase are present in RPE,[14] and although their exact function is unknown, this finding supports the view that a complex system of free radical production and disposal is operating continuously in these cells. The process whereby phagocytized ROS disks are degraded and converted to lipofuscin is poorly understood; however, we suspect that the accumulation of lipofuscin granules in the RPE in senescence[15, 16] and in vitamin E deficiency states[3] is the consequence of failure of some of the free radical protective mechanisms.

The devastating vessel growth in retrolental fibroplasia is initiated by high oxygen concentrations in premature infants. Although a role for vitamin E was considered by early investigators, perhaps other components of the armamentarium that protects tissues from oxygen toxicity should now be examined for clues to the special vulnerability of the immature retinal vascular endothelium.[17]

The gradual yellowing of human lenses with age is now postulated to be due to ultraviolet light–induced free radical alteration of aromatic amino acids (principally tryptophan) of lens proteins.[18] Exposure of normal lenses to ultraviolet light for more than 24 hours results in formation of fluorescent substances with spectral qualities resembling the yellow coloration of old lenses.[19] Also, light-induced free radical oxidation of PUFA present in lens fiber membranes could lead to permeability

changes that initiate cataracts. Normal protection of lens membrane lipids against radiation insults during the long human life-span may be provided by the lens glutathione peroxidase system.[18, 20]

In summary, oxygen toxicity and light damage—two seemingly unrelated phenomena—may in fact have a common mechanism through which they cause cellular destruction, namely, the production of free radicals. Even though ocular tissues have a normal complement of protective mechanisms against free radicals, if any step in the defense system fails, the tissue is unable to cope with the continuous production—by light and/or oxygen—of cytotoxic species. The mechanism of free radical damage in the pathogenesis of disease processes and in senescence is a challenging area for future investigation.

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