Lipid Peroxidation and the Aging Process

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Consistent evidence supports the hypothesis that a progressive accumulation of oxidative damage to important cellular molecules is a fundamental mechanism involved in most senescence-associated alterations. Oxidative damage occurs when free radicals produced within an organism are not completely destroyed by the appropriate endogenous defense systems. Because lipids are a major component of living organisms and probably the first target of free radicals once they are produced, lipid peroxidation might play an important role in initiating and/or mediating some aspects of the aging process. It has been widely demonstrated that there is an age-associated increase in the steady-state concentrations of lipid peroxidation products. However, establishing the involvement of this phenomenon in the pathogenesis of the aging process has not been an easy task. The recent development of more reliable techniques to measure lipid peroxidation, together with more well-defined animal models of aging, should be of great help in future studies in this field. The current evidence for the presence and importance of lipid peroxidation in the aging process is discussed in this review.

Introduction

The oxidation of lipids, which is responsible for the rancidity of fats and oils in general, has been acknowledged since antiquity as a serious problem for food storage. Our ancestors recognized that changes associated with the deterioration of oils or animal fats are typically linked to the development of unpleasant odors and tastes. It was also understood that this phenomenon is secondary to some sort of reaction between the “air” (oxygen) and the product to be preserved. Thus, attempts to avoid this reaction were made during past centuries. However, only in the 1940s were the mechanisms by which lipids react with oxygen elucidated, by scientists in the British Rubber Products Association. Lipid oxidation interested not only chemists and food scientists but also people such as museum curators, who wished to preserve valuable biological and nonbiological materials for posterity. These inquiries formed the basis for the initial interest in the potential role played by lipid oxidation in the progressive and general process of aging.

The aging process, also called senescence, is the most common feature of the postreproductive phase of life. It manifests in all multicellular organisms and is characterized by a progressive reduction in the efficacy of a number of physiological processes. This decline translates to a reduced capacity to maintain homeostatic control of important functions and finally results in the death of the organism. Almost 50 years ago, Deham Harman posited that the aging process is the result of free radical (FR)-mediated damage to tissues or organs. His hypothesis was based on the observation that irradiation of living organisms, a process known to induce the formation of FRs, shortened the organisms’ life-spans and produced changes that resembled senescence (1).

A FR is a chemical species that contains one or more unpaired electrons. We and other forms of aerobic life use oxygen to obtain chemical energy from a large pool of biomolecules (see “The Two Faces of Oxygen”†‡). This fact means that when we oxidize any substrate with oxygen, the oxygen itself becomes reduced and is the source of FRs (also called reactive oxygen species, or ROS). In fact, about 2 to 3% of the oxygen consumed by a cell is converted into FRs (2). Today it is well accepted that FRs are continuously produced in vivo, some accidentally (for example, by spontaneous and random autooxidation reactions, which are not regulated by enzymes) and some deliberately (for example, for phagocyte killing mechanisms and intra- or intercellular signaling). Once produced, FRs promptly react with their surroundings in two possible ways: (i) with another FR, so that their unpaired electrons join to make a covalent bond; or (ii) with a non-FR. The latter possibility is probably the most frequent type of reaction, because the majority of the molecules found in vivo are non-FRs and contain paired electrons (3). Depending on the nature of the substrate attacked, different reactions can occur, including lipid peroxidation, protein oxidation, or DNA oxidation. When a lipid is oxidized by a FR, a peroxide is usually generated, so the phenomenon is called lipid peroxidation (Fig. 1). If the lipid is oxidized with the help of an enzyme (such as cyclooxygenase), the process is called lipid oxidation. Today, however, the two terms are used interchangeably.

Mechanisms by which to inactivate FRs have developed during the course of evolution and provide living organisms with several ways to protect themselves from oxidative attacks. These defense mechanisms include a variety of antioxidant enzymes such as superoxide dismutase (SODI‡), catalase (CAT1†), and...
glutathione reductase (see Nicholls Perspective), to mention just a few. In addition, within the hydrophobic lipid bilayer of cell membranes, certain molecules function as antioxidants, such as vitamin E and \( \beta \)-carotene. Finally, extracellular fluids contain a large set of proteins and low-molecular-mass molecules that can act as antioxidants, including albumin, ceruloplasmin, bilirubin, urate, and ascorbic acid. Despite these protective mechanisms, an imbalance between the production of FRs (oxidants) and the systems that defend against them (antioxidants) sometimes occurs, resulting in oxidative stress. Such stress can lead to irreversible biochemical changes in vital biomolecules and subsequent tissue damage (4).

At the core of the FR theory of aging is the hypothesis that a progressive accumulation of macromolecular oxidative damage is the fundamental cause underlying senescence-associated alterations. Evidence supporting the connection between the aging process and oxidative damage in general, and lipid peroxidation in particular, is discussed below.

### The FR Hypothesis of Aging

One of the most important predictions of this theory is that aging is secondary to the accumulation of oxidative damage. In other words, with time, organisms produce more and more FRs, some of which are not completely neutralized by endogenous antioxidant defense mechanisms. These FRs then react with biomolecules and, as a result, lead to the accumulation of toxic oxidative products. Another possibility is that, with time, the ability of the defense systems to eliminate FRs is reduced, which would also culminate in oxidative stress and the accumulation of toxic oxidative products, even if the amount of FRs produced remained the same. Most of the studies performed in order to test this prediction have amply demonstrated that the second scenario occurs. As a result, there is an age-dependent increase in the steady-state concentrations of oxidatively damaged biological macromolecules, such as lipids, proteins, and DNA, in various organs and tissues (5-10).

In general, it has been shown that tissues such as the brain, heart, and skeletal muscle, which are composed of postmitotic cells, tend to accumulate greater quantities of oxidative damage with aging than do tissues with actively dividing cells (11). Organs can protect themselves from accumulating oxidative damage by increasing their cellular turnover, because newly generated cells have fresh, fully functional, antioxidant machinery. Organs with mainly nondividing cells are particularly vulnerable to the loss of antioxidant systems and cumulative oxidative damage, because their cell turnover is absent or infrequent.

It has also been demonstrated that the mitochondria of older animals produce substantially more FRs than do those of younger animals, for reasons that are not understood. Thus, in a process that escalates during aging, damaged mitochondria would leak more FRs, which would have a negative effect on the mitochondria themselves as well as on the entire cell body. As a result, there would be a depletion of energy and subsequent degeneration of that particular tissue or organ, both of which are hallmarks of aging (12, 13).

Another important piece of evidence that supports the FR theory of aging is the inverse relationship that exists between the average life-span of a species and both the rate of FR generation by mitochondria and the steady-state concentrations of biological macromolecules damaged by FR attack (14-16). In contrast, the issue of whether the rate of aging can be altered by manipulating antioxidant systems by overproducing key enzymes remains controversial. In fact, there are some conflicting results in the literature regarding the relationship between lifespan and the expression levels of different components of antioxidant defense systems (16, 17). In general, it would appear that rates of FR generation and the subsequent accumulation of oxidatively damaged macromolecules are better correlated with the aging process than are the levels of antioxidant defenses.

### Lipid Peroxidation and Aging

The possible relevance of lipid peroxidation to biology and medicine was first considered in the 1950s, as a result of the elucidation of the biochemistry of the reaction between fatty acids and molecular oxygen, together with the development of new technologies. Today, many techniques are available to measure lipid peroxidation (Fig. 2). Most of these methods work well when applied to in vitro systems such as liposomes or microsomes, but there are some limitations when these procedures are applied to more complex biological systems (see (4, 18) for review).

The cell membrane is a target that is particularly rich in lipids, which are easily accessible to FRs in the cell and susceptible to peroxidizing reactions mediated by FRs. For example, FRs react with membrane fatty acids and phospholipid components to form lipid peroxides (Fig. 1), which can induce an irreversible impairment of membrane fluidity and plasticity and thereby lead to irreversible damage to the cell’s integrity. All of these changes are likely to be particularly important in long-lived postmitotic cells, because the rate of lipid turnover in these cells is lower than in dividing cells. Furthermore, some of the breakdown products of lipid peroxidation reactions are believed to contribute to the production of lipofuscin, a structurally heterogeneous yellowish-brown granular pigment that accumulates in the cytosol with age and appears to be a universal correlate of senescence in animals. The accumulation of lipofuscin is particularly evident in postmi-

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§ http://sageke.sciencemag.org/cgi/content/full/sageke;2002/50/re5
toxic cells. For example, up to 7% of the intracellular volume of human myocardial cells derived from 90-year-old individuals might be occupied by this pigment. In contrast, lipofuscin is completely absent or almost undetectable in cells derived from young individuals. Indeed, lipofuscin provides strong evidence to support the premise that lipid oxidative processes occur in vivo and, although not completely proven yet, it has been hypothesized that its accumulation compromises vital cell functions, such as respiration and energy production (19).

Among the different lipid components of the cell, polyunsaturated fatty acids (PUFAs) exhibit the highest sensitivity to FR-mediated attack. In general, the sensitivity of fatty acids to oxidation increases exponentially as a function of the number of double bonds per molecule, because these bonds are relatively prone to attack (20). Thus, the presence of high concentrations of saturated fatty acids—those containing no double bonds in their carbon tails—in cell membranes could be advantageous for cells by reducing their sensitivity to FR-mediated lipid peroxidation. Oxidation of PUFAs leads to the formation of hydroperoxides and endoperoxides, which in turn can undergo fragmentation to yield a broad range of many reactive intermediates, including alkanals, alkenals, hydroxyalkenals, malondialdehyde (MDA), and hydroxynonenal (HNE). These carbonyl compounds and their peroxide precursors are highly unstable and reactive. They are well suited to attacking nucleophilic groups in proteins, an activity that results in irreversible chemical, structural, and possibly functional modifications. Two pathways by which HNE forms protein adducts are shown in Fig. 3. The alteration of amino acids in proteins by the products of lipid peroxidation can result in the formation of a variety of new products, such as MDA-lysine and carboxymethyllysine adducts, by nonenzymatic means. These products can also be deleterious for cell homeostasis (21). For example, they can inhibit cell growth, disrupt microtubules, and irreversibly modify cytoskeleton proteins (22).

Interestingly, it has been demonstrated that the cells of some long-lived animal species, including pigeons and large mammals, contain fatty acids that are relatively saturated, display a low sensitivity to lipid peroxidation, and in general contain a low concentration of lipid oxidation-derived adducts (23-25). Thus, it is evident that at least in some animal models, the degree of fatty acid unsaturation inversely correlates with the maximum longevity exhibited by these species. Increased concentrations of PUFAs might have detrimental effects on cellular function. Examples of such effects include increases in mitochondrial proton leak, increased mitochondrial breakage and dysfunction, and resulting decreases in the control of cellular respiration. These changes would lead to an increase in the FR-mediated formation of lipid peroxidation products, the presence of which is associated with various chronic diseases.

Some studies have suggested a role for fatty acid desaturation as another important factor in the determination of the aging rate. For instance, relative to control animals, the senescence-accelerated mouse prone (SAMP) strain (in which accelerated aging and short life-span are believed to be under complex genetic control) has higher concentrations of PUFAs, such as arachidonic acid and docosahexaenoic acid, and lower concentrations of the relatively saturated linoleic acid in cell membranes derived from the brain (26). Cell membranes from the SAMP mouse also display a higher peroxidizability index, which is determined by an in vitro test that measures how fast a fatty acid or other lipid can be oxidized after an exogenous oxidant is added, compared to the control (26). Among human populations, the Inuit have very low concentrations of arachidonic acid in their plasma phospholipids as a result of a genetic lack of delta-5 desaturase activity (27). The arachidonic acid concentration in their plasma phospholipids remains constant even after these individuals change to a linoleic acid-rich diet (for example, one containing corn oil), which normally would result in a strong increase in arachidonic acid levels. It is known that low delta desaturase activities limit the conversion of dietary linoleic acid to the highly unsaturated fatty acids such as arachidonic or docosahexaenoic acid (28). Interestingly, the Inuit have unusually low incidences of coronary heart disease and other chronic diseases associated with aging (27). All of these facts raise the possibility that variations in desaturase activities can explain part of the differences in the rate of aging in a variety of systems. Low levels of unsaturation in fatty acids in the plasma membrane is a trait of all the long-lived homeothermic (warm-blooded) vertebrates studied thus far (pigeons and humans), relative to their short-lived counterparts (rats and mice), and this feature could be one of the main reasons behind the low rate of aging in these animals (29).

Similarly, a relationship between the rate of aging or life expectancy of several animal species and the degree of fatty acid unsaturation and concentrations of lipid peroxidation products in cells derived from a number of different organs has also been demonstrated. In general, the highest degree of lipid peroxida-
What is the putative mechanism by which FR-mediated oxidative damage of lipids plays a causal role in the aging process? On the basis of the information available to date, it is possible to hypothesize that oxidative damage to the fatty acid constituents of the cell membrane, if not prevented or corrected in time, could result in secondary damage that interferes with important cell functions. For example, such damage to the cell membrane can lead to deleterious effects on protein structure and/or function, which would ultimately culminate in a substantial reduction in cell survival.

Conclusions

Whether changes observed in senescent cells are primary or secondary to lipid peroxidation has not been completely established and will require more experimental work. To prove conclusively that FR-mediated lipid peroxidation plays a causative role in the aging process is a challenging task. It will also be difficult to determine the relative contributions of lipid, protein, and DNA oxidation to the phenomenon of aging.

A difficult general question to be addressed is the following: Is FR-mediated damage to lipids a cause or simply a concomitant phenomenon associated with the aging process? Obviously, this is an important question. Only with a thorough understanding of the mechanisms behind the oxidative damage of lipids will we have the opportunity to develop specific and effective approaches for reducing the impact of all the diseases related to the lipid peroxidation process.

Acknowledgment

I dedicate this article to my wife, Barbara, whose support through all these years has been one of the reasons for my achievements.

References