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Mitochondrial decay in aging

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Abstract

Several mitochondrial functions decline with age. The contributing factors include, the intrinsic rate of proton leakage across the inner mitochondrial membrane (a correlate of oxidant formation), decreased membrane fluidity, and decreased levels and function of cardiolipin, which supports the function of many of the proteins of the inner mitochondrial membrane. Oxidants generated by mitochondria appear to be the major source of the oxidative lesions that accumulate with age. Evidence supports the suggestion that age-associated accumulation of mitochondrial deficits due to oxidative damage is likely to be a major contributor to cellular, tissue, and organismal aging.

Keywords: Mitochondrial function; Proton leakage; Cardiolipin; Aging

1. Introduction

A general decline in physiological function that leads to morbidity and mortality is associated with aging. Various lines of evidence implicate stochastic events as being a fundamental driving force behind this process [1]. We recently reviewed [2] the evidence that sustained damage inflicted by endogenously produced oxidants are the likely cause of the age-related deficits in mitochondrial function. This decline is associated with a generalized physiological decline that is common to all aging organisms. We also have discussed [3] the evidence that oxidation is a major contributor to cellular aging and the degenerative diseases that accompany aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. Also reviewed was the evidence that dietary antioxidants, such as ascorbate, tocopherol, and carotenoids, the main source of which are fruits and vegetables, protect against these degenerative diseases.

Superoxide, H_2O_2, and hydroxyl radicals (the same oxidants produced by radiation) and possibly singlet oxygen are produced continuously at a high rate as a by-product of aerobic metabolism. They damage cellular macromolecules, including DNA [4], protein [5], and lipid [6]. Accumulation of such damage may contribute to aging and age-associated degenerative diseases.

An impressive array of cellular defenses have evolved to battle these reactive oxidants [7]. However, these defenses are not perfect and consequently, cellular macromolecules become oxidatively damaged. The accumulation of these damaged macromolecules is proposed to contribute significantly to aging [3].

Mitochondria appear to constitute the greatest source of oxidants. (1) The mitochondrial electron transport system consumes approx. 85% of the oxygen utilized by the cell. (2) Compared with other oxidant producing systems of the cell (cytochrome *P*-450, various cytosolic oxidases, β-oxidation of fatty acids in peroxisomes, etc.), mitochondria are required for the production of ATP and are present in relatively high numbers in essentially all cells of the body. Cellular energy deficits caused by declines in mitochondrial function can impair normal cellular activities and compromise the cell's ability to adapt to various physiological stresses. We argue that this oxidative damage, and in particular oxidative damage to mitochondria, is a major factor in aging.

2. Oxidation of mitochondrial DNA

Levels of oxidative damage to mtDNA isolated from rat liver or various human brain regions are at least 10-fold higher than those of nuclear DNA [8–10]. This increase correlates with the 17-fold higher evolutionary mutation rate in mtDNA compared to nuclear DNA [11]. The higher levels of oxidative damage and mutation in mtDNA have

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been ascribed to 3 factors: (a) location of the DNA near the inner mitochondrial membrane sites where oxidants are formed, (b) lack of protective histones, and (c) lack of DNA repair activity. Oxidative lesions in mtDNA accumulate as a function of age in human diaphragm muscle [12], human brain [8], and rat liver (Fig. 1) [3]. The amount of oxo^8dG, a biomarker of oxidative DNA damage, in mtDNA in human diaphragm muscle is reported in an 85 year old individual to reach levels of approx. 0.5% of the dG residues in mtDNA. Comparisons of this mtDNA with mtDNA isolated from younger individuals indicate an approx. 25-fold increase with age. A high level of oxo^8dG (0.87% of dG residues) is also observed in mtDNA isolated from regions of the human brain isolated from one individual 90 years of age [8]. The level of oxo^8dG in mtDNA of rat liver shows a 2–3-fold increase in 24-month old rats (less than their maximal lifespan of 30 months) [3]. This less impressive elevation might possibly reflect a decreased accumulation of damage in mitotic vs. post-mitotic cells. The age-associated accumulation of oxidative damage to mtDNA correlates with the level of mtDNA deletions seen in a number of tissues composed of post-mitotic cells (see below; [12]). It is argued that this damage leads to mutations that result in dysfunctional mitochondria. Oxidative damage to brain mtDNA may contribute to the age-dependent increase in the incidence of neurogenerative diseases [8].

3. Oxidation of mitochondrial protein

The accumulation of oxidatively damaged proteins, the extent of which varies within and among tissues, increases markedly with age [5]. As in the case of oxidative damage to DNA an age-associated increase in oxidative damage to mitochondrial protein is observed [13]. The accumulation of oxidized dysfunctional protein with reactive carbonyl groups could lead to inter- and intramolecular cross-links with protein amino groups and cause loss of biochemical and physiological function in mitochondria. Thus the age related accumulation of protein oxidation products in mitochondria may also lead to loss of energy production and increased production of oxidants.

4. Oxidation of mitochondrial lipids

The fluidity of cellular membranes decreases with age [14], a change that may be attributed in part to oxidation of plasma and mitochondrial membrane lipid components. Part of this increased sensitivity to oxidants appears to be due to changes in membrane lipid composition. For example, in the liver microsomal and mitochondrial membrane fractions isolated from rodents, there appears to be a progressive decline in the amount of linoleic acid (18:2). This change is roughly paralleled by an increase in the amount of long-chain polyunsaturated fatty acids (22:4 and 22:5), a subclass of lipids that exhibit a higher degree of unsaturation and are more sensitive to oxidation reactions than linoleic acid [15]. Most of these substitutions (18:2 to 22:4 and 22:5) appear to occur in the fatty acid composition of cardiolipin. Because cardiolipin plays a pivotal role in facilitating the activities of key mitochondrial inner membrane enzymes (see below), it would be expected that changes that increase its susceptibility to oxidative damage would be deleterious to normal mitochondrial function.

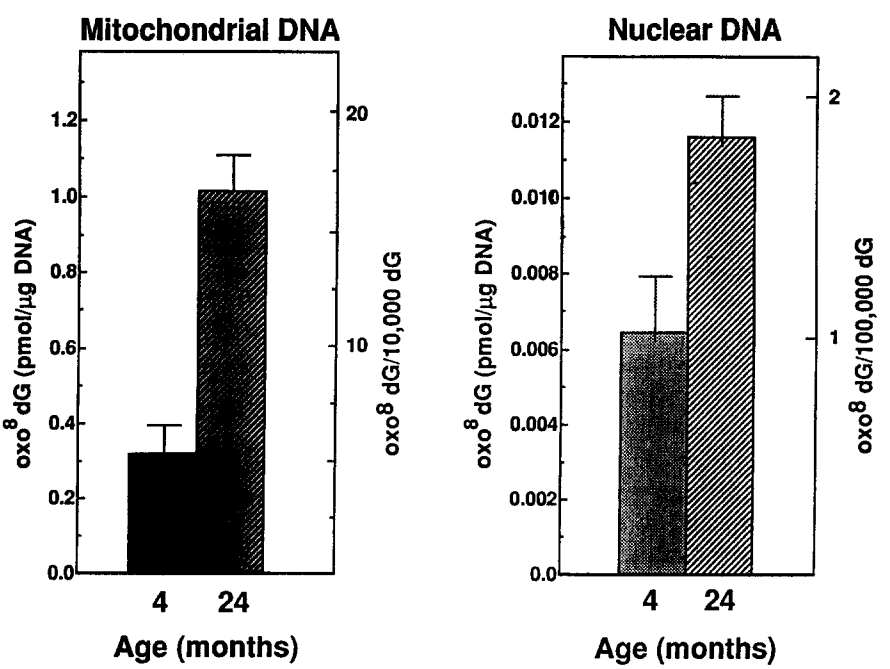

Fig. 1. Steady-state oxidation DNA damage increases with age. 8-Oxo-2′-deoxyguanosine (oxo^8dG) was analyzed [4] in nuclear and mitochondrial DNA from the livers of young and old rats.

We have recently developed a new sensitive and specific assay for malondialdehyde bound to protein [16]. With this assay it can be demonstrated that protein bound malondialdehyde accumulates with age (Fig. 2).

The age-dependent accumulation of lipids that are more prone to peroxidation may also, following peroxidation, increase the rigidity (or decrease the fluidity) of cell membranes. Mitochondria appear to account for essentially all the net loss of water that occurs with age in certain tissues (liver and heart) [17,18], which is consistent with the age-associated increase in membrane rigidity observed in this organelle. Similarly, decreases in lateral diffusion of plasma membrane proteins (e.g., receptors) appear to be associated with a general decline in signal transduction that is commonly observed in aging organisms.

Phospholipase A_2 appears to be important for repair of oxidatively damaged lipids. Phospholipase A_2 activity in the inner mitochondrial membrane increases in response to conditions associated with increased oxidant production, such as bacterial endotoxin treatments [19]. Increases in inner mitochondrial phospholipase A_2 activity are also observed in mitochondria isolated from rats that are fed fish oil [20], or a diet that is deficient in vitamin E [21], two diets associated with increased lipid peroxidation. Efficient membrane antioxidants such as ubiquinol and its synthetic derivatives inhibit release of fatty acids catalyzed by phospholipase A_2 [22], presumably by inhibiting oxidation of lipids. Physiological conditions such as hypothyroidism or hibernation, which are associated with reduced mitochondrial oxygen consumption, are associated with a marked decline in phospholipase A_2 activity [23]. These observations support the suggestion that phospholipase A_2 is a repair enzyme that catalyzes the removal of oxidized lipids in membranes [24]. Without such a repair activity peroxidized lipids could accumulate, the consequence of which might include increased membrane permeability and loss of mitochondrial respiratory control.

5. Bioenergetics

The components of the electron transport chain, which catalyze the phosphorylation of ADP to ATP, work as an integrated system comprised of a total of 5 protein complexes. mtDNA encodes 13 of the proteins and nuclear DNA encodes approx. 60. Complexes I–IV are involved in the oxidation of NADH, electron transport, and the generation of an electrochemical gradient. This electrochemical gradient, which is created by pumping protons across the inner mitochondrial membrane, is utilized by ATP synthase (complex V) as a source of energy. Relevant to mitochondrial function is the efficiency of electron movement through the electron transport chain and its coupling to oxidative phosphorylation to produce ATP. The coupling efficiency can be measured experimentally by determining the ratio of ATP production to molecular oxygen consumed (ADP/O), and whether the mitochondria are in State 3 or State 4. State 3 represents a condition where oxidative phosphorylation is not rate limited by ADP concentration. State 4, a condition where the level of ADP limits oxidative phosphorylation, is associated with a reduced respiratory chain, leading to increased formation of $O_2 \cdot^-$ byproduct.

Temporary or sustained loss of mitochondrial function and ATP production can have a major impact on the fidelity of cellular defenses and repair processes. This may result in increased mutational load, increased accumulation of dysfunctional cellular macromolecules, and a decreased capacity to mount an appropriate stress response when challenged. Probable age associated loss of function in mitochondria is suggested by the evidence of increased mtDNA deletions [25,26] and point mutations [27,28], increased oxidative damage to mtDNA [3,8,12] increased levels of aberrant forms of mtDNA [29,30], formation of mtDNA-protein cross-links [31] increased production of mitochondrially-derived oxidants [32–36] decreased State

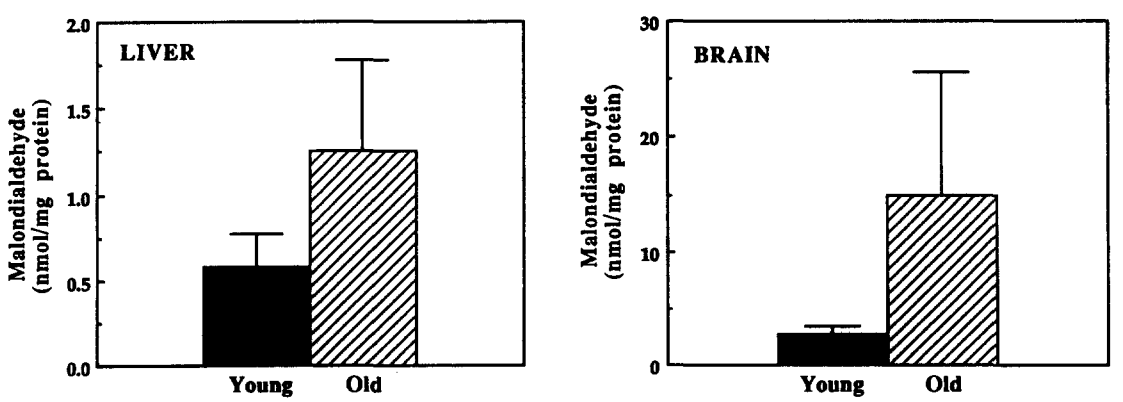

Fig. 2. Malondialdehyde accumulation in male rat (Fischer 344) mitochondria as a function of age. Young (3 months) rats were compared to old (26–31 months).

3/State 4 ratio [37,38], decline in activities of complexes I, II and IV [39–42], and age-related decreases of mitochondrial cytochrome oxidase in post-mitotic tissues [43]. Marked changes in mitochondria with age have been observed histologically, including enlargement, matrix vacuolization, shortened cristae and loss of dense granules [44]. As only about half of these enlarged mitochondria can be recovered from old animals [44] it is quite possible that differences in the function of mitochondria isolated from old vs. young animals are underestimated by this selective loss and may be one reason for the apparent lack of age-associated biochemical changes in this organelle (reviewed in [37]). Along with the histological changes cited above, the potential for lipid peroxidation (the 'peroxidizability index') in the inner mitochondrial membrane increases [45], making the mitochondria more susceptible to damage by oxidants. Furthermore, the decreased content of 18:2-containing lipids, which are optimal for cardiolipin interactions with proteins of the inner mitochondrial membrane [46], may account for the decreased State 3/State 4 ratio, and increased $O_2 \cdot^-$ and H_2O_2 formation that has been observed in some tissues with age. These changes, in turn, can contribute to increased loss of efficiency in mitochondrial function.

6. Mitochondrial DNA mutations and aging

mtDNA defects can lead to mitochondrial dysfunction; some of these defects are genetically inherited and have been shown in some instances to be associated with an extensive amount of mtDNA deletions (30–80% of all mtDNA) or point mutations resulting in energy deficits and compromised tissue function [45]. mtDNA deletions, many of which are produced because of illegitimate recombinational events at direct repeat sequences are particularly prevalent in post-mitotic tissues [47]. Associated with these deletions are myopathies and increased susceptibility to neurodegenerative disorders.

The same type of deletions and point mutations in mtDNA that cause inherited myopathies are also observed to increase with age [48]. The age-associated increase in the level of any of the common deletions (e.g., mtDNA 4977, mtDNA 7436, mtDNA 10,422) produced spontaneously is low ($< 0.1\%$ vs. 30–80% for inherited cases). While the effect of this low level of deletions may not be significant, it is postulated that these deletions represent only a small portion, 'the tip of the iceberg' [47], of the multitude of deletions and point mutations that might exist and accumulate with age. It is plausible that the accumulation of all mtDNA defects could account for the age-related deficits in mitochondrial bioenergetic capacity and function.

The role of oxidants in the formation of mtDNA deletions is supported by the observation that doxorubicin, a compound that stimulates mitochondrial oxidant production, creates a marked elevation in mtDNA deletions in cardiac tissue; this effect is blocked by ubiquinone (Coenzyme Q_{10}) [49], a key component of the mitochondrial electron transport system whose reduced form, ubiquinol, exhibits antioxidant properties [50]. The age-associated accumulation of the common deletion mtDNA 4977 also appears to correlate with oxygen consumption [47,51] as well as functional workload [52]. This and other mtDNA deletions have been postulated to be responsible for the degeneration of neurological function, cardiovascular function, and muscle movement that are common in older individuals [53].

Studies that have examined the content of cytochrome oxidase in mitochondria show a progressive and random loss in this enzyme activity [43,54] which correlates well with the age-associated decline in mtRNA synthesis [55]. A study of human diaphragm muscle indicates that cytochrome *c* oxidase decreases markedly beyond the seventh decade of life [54]. Examination of various muscle tissues (extraocular muscles, human diaphragm, skeletal muscles), brain, liver, heart, and lung [12,25,26,47,56,57] indicate age-associated increases in mtDNA deletions. These deletions are proposed to create tissue bioenergy mosaics [26,58] that may account for losses in bioenergetic capacity. This has been shown to occur with age in skeletal muscle [59] and in liver [60].

7. Mitochondrial compensatory mechanisms

The loss of functional mitochondria with age appears to be compensated in part by the increased workload of the remaining intact population of mitochondria [61]. The increase in senescent tissue of mtDNA copy number [62] supports the idea of an adaptive mechanism designed to restore mitochondrial function. These changes may account for the apparent lack of effect of aging on the level of adenine nucleotide levels observed in cells of aged organisms.

Thus, the increases in either mtDNA copy number [62] or increases in the expression of nuclear encoded proteins for oxidative phosphorylation [52] may be feedback mechanisms that compensate for mitochondria harboring defective proteins or mtDNA. The result of such a mechanism is to allow a cell to adapt to a localized loss of mitochondrial function. Compensatory mechanisms in the fully functional cells mask the inefficiencies of their dysfunctional neighbors but in doing so increase their workload, their energy expenditures, and the probability of incurring damage and loss of function.

8. Imbalances in the electron transport chain produce increased superoxide and hydrogen peroxide

Damage to inner membrane proteins comprising the electron transport chain can alter the efficiency of electron

transport. Imbalances in the stoichiometry of functional electron transport proteins is proposed to lead to a leakage in the flow of electrons to the terminal electron acceptor, cytochrome oxidase [63]. The decreased age-related expression of cytochrome oxidase in tissues such as the heart, liver, and brain are of particular relevance. Furthermore, alteration in protein conformation due to direct oxidative damage or through DNA mutation may cause inefficient transfer of electrons through the electron transport chain. This would increase the likelihood of superoxide formation. Treatment of submitochondrial particles with glutaraldehyde, increases $O_2 \cdot^-$ and H_2O_2 production, presumably by inducing crosslinks between proteins and lipids of the inner mitochondrial membrane [64]. Crosslinks of inner mitochondrial membrane proteins by oxidants, or reactive aldehydes generated from lipid peroxidation, may also result in increased $O_2 \cdot^-$ and H_2O_2 production, thus further increasing the damage that can lead to mitochondrial dysfunction.

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