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Critical Review

Mitochondrial Oxidative Stress Plays a Key Role in Aging and Apoptosis

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Summary

Harman first suggested in 1972 that mitochondria might be the biological clock in aging, noting that the rate of oxygen consumption should determine the rate of accumulation of mitochondrial damage produced by free radical reactions. Later in 1980 Miquel and coworkers proposed the mitochondrial theory of cell aging. Mitochondria from postmitotic cells use O_2 at a high rate, hence releasing oxygen radicals that exceed the cellular antioxidant defences. The key role of mitochondria in cell aging has been outlined by the degeneration induced in cells microinjected with mitochondria isolated from fibroblasts of old rats, especially by the inverse relationship reported between the rate of mitochondrial production of hydroperoxide and the maximum life span of species. An important change in mitochondrial lipid composition is the age-related decrease found in cardiolipin content. The concurrent enhancement of lipid peroxidation and oxidative modification of proteins in mitochondria further increases mutations and oxidative damage to mitochondrial DNA (mtDNA) in the aging process. The respiratory enzymes containing the defective mtDNA-encoded protein subunits may increase the production of reactive oxygen species, which in turn would aggravate the oxidative damage to mitochondria. Moreover, superoxide radicals produced during mitochondrial respiration react with nitric oxide inside mitochondria to yield damaging peroxynitrite. Treatment with certain antioxidants, such as sulphur-containing antioxidants, vitamins C and E, or the Ginkgo biloba extract EGb 761, protects against the age-associated oxidative damage to mtDNA and the oxidation of mitochondrial glutathione. Moreover, the EGb 761 extract also prevents changes in mitochondrial morphology and function associated with aging of the brain and liver.

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THE FREE RADICAL THEORY OF AGING: A KEY ROLE FOR MITOCHONDRIA

One of the most relevant theories raised to explain aging is the free radical theory of aging, which was first proposed by Harman in 1956 (*1*). According to Harman, oxygen-derived free radicals are responsible for the age-associated impairment at the cellular and tissue levels.

At present, a great deal of experimental evidence supports the free radical theory of aging, especially the extension of life span obtained by increasing the antioxidant defense as well as the inverse relationship between the rate of reactive oxygen species (ROS)[1] production and the maximum life span of species (*2–4*). Thus, administration of antioxidants can increase the mean life span of flies (*5*, *6*). Orr and Sohal (*7*) have recently found that simultaneous overexpression of copper-zinc superoxide dismutase and catalase genes in transgenic *Drosophila* extended their mean and maximum life span; furthermore, these transgenic flies exhibited a delayed loss of physical performance and a less oxidative damage to proteins.

Age-related declines of cognitive function and motor skills are associated with oxidative protein damage within different regions of the brain (*8*). Moreover, administration of the spin-trapping agent *N-tert*-butyl-α-phenylnitrone (*9*) or dietary restriction (*10*) decreased the oxidative damage to protein in the brains of rodents, with a concurrent improvement in age-related behavioral deficits.

More than 90% of the oxygen used by aerobic cells is consumed in mitochondria and ~1–2% of oxygen used by mammalian mitochondria in state 4 forms not water but oxygen-activated species (*11*, *12*). Thus, oxygen free radicals and hydroperoxides are generated continuously in the mitochondrial respiratory chain (*11*, *12*) and they, particularly the hydroxyl radical, can cause oxidative damage to proteins, lipids, and DNA.

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[1] Abbreviations: GSH, reduced glutathione; mtDNA, mitochondrial DNA; 8-oxodG, 8-hydroxydeoxyguanosine; PT, permeability transition; ROS, reactive oxygen species.

Harman again was the first to suggest, in 1972, that mitochondria might be the biological clock in aging, noting that the rate of oxygen consumption should determine the rate of accumulation of mitochondrial damage produced by free radical reactions (*13*). Later, in 1980, Miquel and coworkers proposed the mitochondrial theory of cell aging (*14*), which suggests that cellular senescence is a by-product of oxy-radical attack on the mitochondrial genome of fixed postmitotic cells. Mitochondria from postmitotic cells use O_2 at a high rate, hence releasing oxygen radicals, which exceed cellular antioxidant defences (*15*). The key role of mitochondria in cell aging has been outlined by the degeneration induced in cells microinjected with mitochondria isolated from fibroblasts of old rats, but especially by the inverse relationship reported between the rate of mitochondrial hydroperoxide production and the maximum life span of species (*3*, *4*).

The mitochondrial respiratory chain generates superoxide anions, which are converted to hydrogen peroxide within mitochondria either spontaneously or by Mn-superoxide dismutase; the hydrogen peroxide then is released by the mitochondria to the external medium (*17*). Studying different mammalian species, Sohal and coworkers found that mitochondria from shorter-lived species produced greater amounts of hydroperoxide than those from longer-lived species (*18*, *19*).

The "rate of living" theory of aging proposes an inverse relationship between the rate of oxygen consumption and the maximum life span of species (*20*). This theory explains the differences in maximal life span potential among many but not all species. Exceptions to this theory are birds and primates, who exhibit at the same time both high oxygen consumption and high longevity (*21*). The explanation for this paradox is that mitochondrial production of hydroperoxide is not proportional to oxygen consumption (*11*, *21*, *22*). Indeed, Barja and coworkers (*21*) as well as Ku and Sohal (*23*) reported that hydroperoxide production by mitochondria is less in pigeon than in rat, whereas oxygen consumption is higher in pigeon than in rat (*21–23*). Thus, mitochondria from birds use oxygen more efficiently and exhibit less free radical leakage through the respiratory chain (*4*). Hence, the rate of ROS production, and not merely the rate of oxygen consumption, may be considered a determinant of the maximal life span of species.

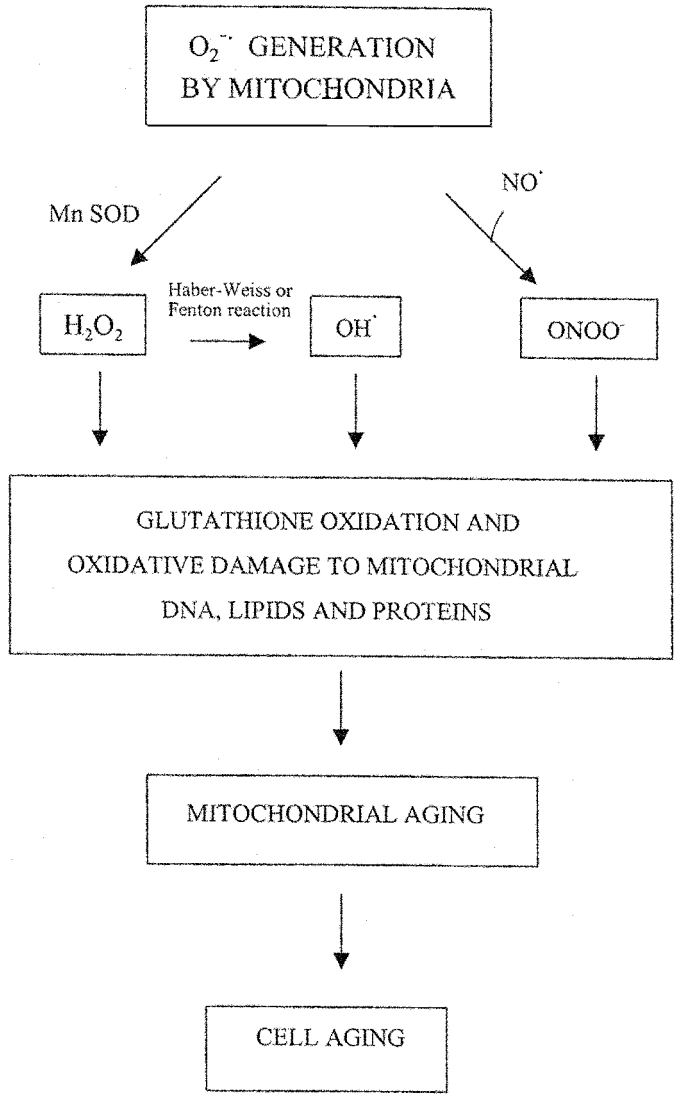

Figure 1. Mitochondrial aging as a model of chronic oxidative stress (from Sastre et al. 2000, *Free Radical Res.* 32, 189–198). MnSOD = Mn-dependent superoxide dismutase; $O_2^{\cdot-}$ = superoxide radical; NO· = nitric oxide; $ONOO^-$ = peroxynitrite; OH· = hydroxyl radical.

MITOCHONDRIAL AGING AS A MODEL OF CHRONIC OXIDATIVE STRESS

The free radical theory of aging assumes that cellular aging is associated with oxidative stress, which was defined by Sies as a disturbance in the balance between pro-oxidants and antioxidants, in favor of the former (*24*). The continuous generation of ROS by mitochondria throughout cell life produces an age-related "chronic" oxidative stress that plays a key role in cellular aging. It is now well established that mitochondrial DNA, proteins, and lipids undergo oxidative damage during aging (see Fig. 1) (*25–29*).

DNA damage has been observed in a wide range of mammalian cell types exposed to oxidative stress (*30*). This damage includes single- and double-strand breaks, deletions, base changes, oxidative damage, and even chromosomal aberrations. The major molecular mechanisms involved are direct reaction of hydroxyl radicals and carbonyl compounds with DNA and activation of nucleases (*30*). Superoxide and H_2O_2 do not react with DNA unless transition metal ions are present to allow hydroxyl radical formation. The hydroxyl radical may attack deoxyribose, purines, and pirimidines, giving rise to numerous products, such as 8-hydroxydeoxyguanosine (8-oxodG), thymidine glycol, and 8-hydroxyadenosine (*30*).

Mitochondrial DNA (mtDNA) is specially susceptible to oxidative damage and mutation because it lacks protective histones (*31*) and is close to the ROS generated continuously by mitochondria. Thus, 8-oxodG formation in mtDNA increases with increasing rates of hydroperoxide production by mitochondria (*32*). Suter and Richter have reported that oxidized bases are present to a moderate extent in the 16.3-kb mtDNA molecules but in very prevalent in the mtDNA fragments (*33*). These results, together with the finding of endonucleases which recognize oxidative damage to mtDNA, demonstrate the existence of a mtDNA repair system (*33–35*).

In accordance with the mitochondrial theory of aging, Barja and Herrero have recently found that oxidative damage to mtDNA is inversely related to the maximum life span of mammals, whereas oxidative damage to nuclear DNA does not correlate with maximum life span (*36*). Moreover, several studies have reported both that the amounts of oxidative damage to mtDNA are several times greater than those of nuclear DNA and that mtDNA mutates several times more frequently than nuclear DNA (*25*, *33*, *36*, *37*). However, Anson et al. (*38*) indicated recently that oxidative damage to mtDNA has been overestimated when measured in mtDNA purified from isolated mitochondria. In fact, they found that 8-oxodG concentrations were similar in nuclear DNA and mtDNA when the latter was obtained without mitochondrial isolation. Using a procedure we reported (*27*) for isolating mtDNA without having to isolate mitochondria measured 8-oxodG concentrations that were three- to ninefold higher in mtDNA than in nuclear DNA in all eight species studied (*36*). Nevertheless, the various methods used so far to isolate mtDNA and to measure its oxidative damage should be reevaluated to confirm these findings.

Oxidative lesions of mtDNA accumulate with age in human and rodent tissues (*27*, *30*, *39*). Hence, the mtDNA repair system cannot cope with the ROS generated throughout cell life in mitochondria. Point mutations and deletions in mtDNA are seen in tissues from old animals (*41–44*). Deletions in mtDNA increases >10,000-fold with age in humans (*45*). In accordance with the hypothesis of Miquel et al., the most mtDNA deletions are found late in life in postmitotic tissues such as brain, heart, and muscle (*45*). Point mutations and aberrant forms in mtDNA of postmitotic cells also are associated with age-related degenerative diseases (*46*, *47*).

A peculiar characteristic of the age-associated mtDNA deletions is their mosaic distribution (*45*). This implies a focal distribution of the deletion even in the same tissue, with some cells having a high level of the deletion and some only a little. Thus, a difference of two to three orders of magnitude was observed for the 4977-bp deletion—also known as the common deletion—in different brain regions (*48*, *49*).

The impairment of mtDNA may affect transcription of mitochondrial genes (*50*). Indeed, an age-related decrease in the amounts of mitochondrial transcripts in some rat tissues and in *Drosophila* has been reported (*51*, *52*). Furthermore, because mtDNA has no introns, any mutation will affect a coding DNA sequence (*31*). Indeed, Lezza et al. (*53*) found a correlation between the decrease in the oxidative phosphorylation capacity and the increase in the mtDNA common deletion during aging. Thus, it has been suggested that mtDNA mutations might be important contributors to aging and neurodegenerative diseases (*15*, *37*, *54*).

Mitochondrial reduced glutathione (GSH) plays a key role in protecting against oxidative damage to mtDNA. Indeed, the oxidative damage to mtDNA that occurs during aging is directly related to oxidation of mitochondrial glutathione (*27*). Glutathione oxidation increased with age in mitochondria from liver, kidney, and brain of rats (*27*); moreover, the increase was much higher in mitochondria than in whole cells. These results support the idea that mitochondria are a major source of free radicals in aging (*14*, 22, *55*, *56*) and emphasize the relevance of mitochondria as primary targets of the oxidative damage associated with aging (*14*).

A change in the glutathione redox status would indicate that mitochondrial antioxidant systems were not able to cope with the oxidant species generated throughout the life of the cell. Therefore, glutathione oxidation may occur before the oxidative damage to other mitochondrial components and might be an early event in the chronic oxidative stress associated with mitochondrial aging. This points out the importance of cells maintaining an adequate GSH status to protect against oxidative damage to important molecules such as DNA.

The role of protein damage in cell aging became apparent when catalytically less active or inactive forms of some enzymes were found to accumulate during aging (*57–59*). Posttranslational modifications seem to be responsible for this accumulation of inactive proteins (*60*). Most of these modifications may be due to oxygen radical–mediated oxidation of enzymes, which is a key step in protein turnover (*61–63*). Oxidative damage appears to occur selectively in certain mitochondrial proteins. For example, mitochondrial aconitase, an enzyme of the citric acid cycle, is reported to be a specific target of oxidative damage during aging of houseflies (*64*).

Old animals have greater amounts of polyunsaturated fatty acids in their cells than do young ones (*25*, *65*), making their membranes more susceptible to oxidative damage (*25*). Furthermore, the maximum life span of species may depend on the sensitivity of mitochondrial lipids to oxidative damage. Thus, analysis of the fatty acids in liver mitochondria from eight mammalian species has revealed that the total number of double bonds and the susceptibility of mitochondrial membrane lipids to peroxidation are inversely correlated with maximum life span (*66*). Thus, as pointed out by Barja and coworkers (*67*), a low degree of fatty acid unsaturation seems to be a characteristic of long-lived animals, regardless of their metabolic rates.

An important change in mitochondrial lipid composition is the age-related decrease found in cardiolipin content. Indeed, cardiolipin decreases with age in heart, liver, and nonsynaptic brain mitochondria (*68–70*). Because cardiolipin is required for optimal catalytic activity of inner mitochondrial enzymes

(*71*), modifications in its composition may be involved in the age-related decline of mitochondrial respiratory chain activities (*25*, *70*).

As pointed out recently by Wei (*72*), a vicious cycle appears to operate in mitochondria in aging. The concurrent enhancement of lipid peroxidation and oxidative modification of proteins in mitochondria further increases mutations and oxidative damage to mtDNA in the aging process (*72*). The respiratory enzymes containing the defective mtDNA-encoded protein subunits may thus increase the ROS production, which in turn would aggravate the oxidative damage to mitochondria (*72*).

Superoxide radicals produced during mitochondrial respiration also react with nitric oxide inside the mitochondria to yield damaging peroxynitrite (*73*, *74*). Furthermore, mitochondria are themselves a source of NO, which may increase the formation of superoxide radicals and hydrogen peroxide by mitochondria (*75*). Future research is needed to elucidate the role of mitochondrial nitric oxide and peroxynitrite in age-associated oxidative stress.

OXIDATIVE STRESS CAUSES CHANGES IN MITOCHONDRIAL FUNCTION AND MORPHOLOGY IN AGING

Oxidative stress is involved in age-associated deficits in mitochondrial function as well as in changes in mitochondrial morphology (*25*, *29*). The reported age-related decreases in membrane potential of brain and liver mitochondria (*28*, *29*) may reduce the energy supply in old cells, given that the mitochondrial membrane potential is the driving force for ATP synthesis.

An acute oxidative stress causes inhibition of mitochondrial respiration (*77*), which affects the mitochondrial membrane potential. Moreover, hyperoxia reduces the mitochondrial membrane potential in microvascular cells (*78*). Hence, the oxidative stress associated with aging may be responsible, at least in part, for the age-related impairment in mitochondrial membrane potential and respiratory activity. Indeed, intracellular concentrations of peroxide increase with age in whole cells (*28*, *76*), which correlates with parallel changes in peroxide generation by isolated mitochondria (*28*, *29*, *79*). Most likely, the accumulation of peroxides in whole cells in aging comes from the continuous peroxide generation by mitochondria throughout the cell life, although we cannot rule out that other structures, such as peroxisomes, may also have a role.

On the other hand, mitochondrial morphology is important because changes in mitochondrial ultrastructure modulate mitochondrial function (*80*). Indeed, volume-dependent regulation of the packing of matrix proteins modulates metabolite diffusion and, in turn, mitochondrial metabolism (*80*). Enlargement, matrix vacuolization, and altered cristae have been found in mitochondria from old animals by electron microscopy and flow cytometry (*28*, *29*, *81*, *82*). Alterations of mitochondrial cristae in old mitochondria may be responsible for the age-related impairment in mitochondrial membrane potential we have found.

Acute oxidative stress is well known to cause mitochondrial swelling (*83*). Thus, age-associated chronic oxidative stress may be the cause, at least in part, of mitochondrial swelling. Furthermore, changes in mitochondrial morphology and function seem to occur in a correlated way during aging.

Several studies have shown a decline in activities of complexes I, II, and especially IV (*26*). Moreover, the respiratory activity of isolated mitochondria decreases with age in liver, skeletal muscle, and brain (*84–86*). Age-related decreases in the activities of mitochondrial anion carrier proteins—such as the phosphate carrier and ATP/ADP translocation in liver mitochondria (*87*, *88*) and Ca^{2+}, adenine nucleotide, and pyruvate carriers in heart mitochondria (*68*, *82*, *90–92*)—have also been reported.

In studying biochemical pathways that depend on mitochondrial function in isolated hepatocytes, we found that gluconeogenesis from lactate plus pyruvate, but not from glycerol or fructose, decreases during aging (*28*). Gluconeogenesis from lactate involves mitochondria, whereas that from glycerol or fructose does not. The slower rate of gluconeogenesis from lactate plus pyruvate is the result of impaired transport of malate across the mitochondrial membrane by way of the dicarboxylate carrier (*28*). Furthermore, posttranscriptional modifications appear to be involved in the age-related impairment of this carrier, because its gene expression does not change with age (*28*).

Nevertheless, the fact that respiratory activity and some mitochondrial carriers are impaired in aging does not necessarily mean that all mitochondrial functions are affected by aging. For example, the rate of urea synthesis in hepatocytes does not change with age (*28*).

An increased generation of oxygen free radicals may be responsible for the decline in the activity of mitochondrial membrane proteins, such as metabolite carriers and respiratory chain complexes. In fact, exposure of mitochondria to free radicals is known to impair the mitochondrial inner-membrane proteins (*83*) and inhibit mitochondrial respiration (*16*).

Damage to mitochondrial electron transport may be an important factor in the pathogenesis of neurodegenerative diseases, such as Parkinson disease, Alzheimer disease, and amyotrophic lateral sclerosis (*93*). Activation of excitatory amino acid receptors causes enhanced production of NO and superoxides, which can lead to the generation of peroxynitrite (*94–96*). NO and especially peroxynitrite are thought to mediate mitochondrial damage in neurodegenerative disorders, because they can inhibit components of the neuronal mitochondrial respiratory chain (*93*, *97*).

ROS and NO are important physiological modulators of mitochondrial functions but may damage mitochondria when present in excessive amounts (*98*, *99*). NO inhibits respiration reversibly at cytochrome *c* oxidase (*100*, *101*), but peroxynitrite inhibits respiration irreversibly at complexes I–III (*102*) and also at cytochrome oxidase (*103*). Peroxynitrite would be the reactive intermediate accounting for NO-dependent inactivation of electron transport components and ATPase in living cells and tissues (*104*). Further research is needed to clarify the role of

peroxynitrite on mitochondrial aging, especially on the age-related decrease in cytochrome oxidase activity.

ANTIOXIDANTS PREVENT AGE-ASSOCIATED MITOCHONDRIAL OXIDATIVE STRESS

The free radical theory of aging proposed by Harman (*1*) is especially attractive because it provides a rationale for intervention; that is, administration of antioxidant may slow the aging process. In 1979, Miquel and Economos were the first to show that administration of thiazolidine carboxylate increases the vitality and life span of mice (*5*). Later, Furukawa et al. (*105*) reported that oral administration of glutathione protects against the age-associated decline in immune responsiveness. More recently, we found that administration of some sulfur-containing antioxidants protected against the age-associated depletion of glutathione in mouse tissues and also partially prevented the age-related decline in neuromuscular coordination (*6*). These antioxidants also increased the mean life span of *Drosophila* (*6*).

Recently, we have investigated the protective effect of a standardized extract from dried leaves of *Ginkgo biloba* (EGb 761) on the age-associated oxidative damage to mtDNA (*29*). EGb 761 is a mixture of flavonoids, heterosides, and terpenes (*106*). The antioxidant action of this Ginkgo biloba extract is attributable to its components, the flavonol glycosides, which are known for scavenging superoxide anions as well as hydroxyl and peroxyl radicals (*107*, *108*). Because of their ability to interact with and penetrate the lipid bilayers, flavonoids also prevent lipid peroxidation in the membranes (*109*).

Oral administration of EGb 761 to rats for 3 months was able to prevent the oxidative damage to mtDNA that occurs in liver and brain in aging (*29*). Treatment with EGb 761 also protected against the oxidation of mitochondrial glutathione and the age-related increase in peroxide generation by mitochondria (*29*). Hence, EGb 761 prevents the chronic oxidative stress associated with mitochondrial aging in rats.

In addition, treatment with EGb 761 prevented age-associated impairments in mitochondrial morphology and respiratory function. Indeed, this treatment prevented the changes in size and structural complexity that occur in brain and liver mitochondria during aging (*29*). It also prevented the decrease in energy status under state 4 that occurs in liver and brain mitochondria from old rats (*29*). Our results suggest that EGb 761 exhibits beneficial effects on mitochondrial aging by preventing the chronic oxidative stress associated with this process.

We have also found that certain antioxidants, such as thiazolidine carboxylate derivatives or vitamins C and E protect against mitochondrial glutathione oxidation and mtDNA oxidative damage associated with aging (*27*). Moreover, late-onset administration of certain sulphur-containing antioxidants, such as GSH or a thiazolidine carboxylate derivative, is able to prevent not only the age-related oxidative damage to mtDNA in brain but also the impairment in physiological performance, particularly motor coordination, that occurs upon aging (see Fig. 2)

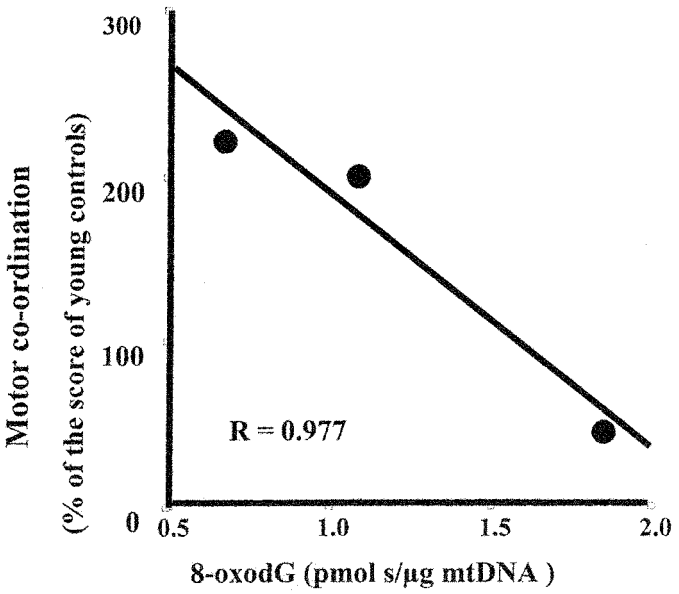

Figure 2. Inverse relationship between motor coordination and oxidative damage to brain mitochondrial DNA in mice. (From Pallardó et al. [*110*]). Points are average for $n = 3$–4 values. The vertical scale indicates the percentage of the score for motor coordination in old mice compared with the score for 12-month-old mice.

(*110*). Thus, we found an inverse relationship between motor coordination and oxidative damage to brain mtDNA in mice. To pursue studies in humans, the practical importance of an effective antioxidant treatment that can be started late in life should be emphasized.

The facts reported here underline the role of oxidative stress, and particularly oxidative damage to mtDNA, in aging at the level of tissue and of the whole organism. Hence, experimental evidences again support Miquel's hypothesis of the key role of mitochondrial oxidative damage in the aging process (*15*) as well as Sohal's hypothesis of the rate of prooxidant generation as a key factor in the rate of aging (*55*).

In conclusion, administration of certain antioxidants—such as GSH, thiazolidine carboxylate derivatives, vitamins C and E, or the *Ginkgo biloba* extract EGb 761—may prevent or delay the oxidative stress and physiological impairment associated with aging. Nevertheless, further studies on dietary supplementation with antioxidants need to be carried out, especially epidemiological studies.

ROLE OF MITOCHONDRIA IN APOPTOSIS; RELEVANCE IN AGING

Mitochondria are key mediators of apoptosis. Kroemer and colleagues (*111*) found that the permeability transition (PT) of mitochondria specifically increases during apoptosis. Permeability transition involves the opening of the so-called PT pores, which are identical to the mitochondrial megachannels located at

the inner–outer membrane contact sites. Kroemer and coworkers (*112*) showed that isolated mitochondria can induce the digestion of nuclear DNA in a cell-free system when PT is activated. In addition, inhibiting PT blocks apoptosis. Thus, mitochondrial PT is a critical step in apoptosis (*112*). Indeed, opening of the mitochondrial PT pores causes release of apoptogenic factors—such as cytochrome *c*; procaspases 2, 3, and 9; and the recently discovered apoptosis-inducing factor—from the intermembrane space (*113*, *114*).

We have recently found that mitochondrial oxidative stress is an early event in apoptosis (*115*). In fact, oxidation of mitochondrial glutathione occurs before fragmentation of the DNA. Hence, mitochondrial oxidative stress may cause mitochondrial PT. We have also found a decrease in mitochondrial membrane potential from apoptotic fibroblasts and an increase in the peroxide content of apoptotic fibrobasts (*115*).

Mitochondria are at the same time the target and the source of ROS (*116*). Mitochondrial dysfunction induced by superoxide, NO, and the consequent production of peroxynitrite plays a pivotal role in neuronal apoptosis or in neurotoxicity induced by several insults (*74*, *94*, *100*, *117*, *118*). Thus, cytochrome *c* release by mitochondria is involved in NO-induced neuronal apoptosis (*119*), and prevention of mitochondrial PT by cyclosporin A protects cells against apoptosis induced by amyloid β-peptide or NO-generating agents (*74*). Exposure to the parkinsonian neurotoxin 1-methyl-4-phenylpyridium and NO simultaneously causes cyclosporin A–sensitive mitochondrial calcium efflux and depolarization (*120*). Hence, NO may induce apoptosis by triggering mitochondrial PT in several cell types, such as neurons and myeloid cells (*121*, *122*). Neuronal apoptosis induced by amyloid β-peptide or NO was prevented by antioxidants such as glutathione or overexpression of mitochondria-localized manganese superoxide dismutase (*74*).

Nevertheless, new evidence suggests that at least two independent cellular pathways may induce apoptosis: one, which requires the presence of mitochondria, and the other, which directly involves the action of specific proteases. This model fits with the contradictory data available on apoptosis research.

There are few reports of the effect of aging on apoptosis, but it has been proposed that the efficiency of apoptosis may correlate with the rate of aging. Experimental studies on rats have suggested that apoptotic cell death provides protective mechanisms by removing senescent or damaged cells that might undergo neoplastic transformation (*123*).

Livers from old rats show a higher in situ rate of apoptosis. Using the dietary restriction model, which is known to retard aging, Muskhelishvili et al. showed (*124*) that tumor incidence in liver may be related to the intrinsic rate of apoptosis, with diet-restricted animals exhibiting a higher rate of apoptosis. Those authors concluded that increased apoptotic activity in livers from old animals was a cellular mechanism of defense against neoplasic degeneration.

On the other hand, some common features of apoptotic cells and of cells from old animals include an increased mitochondrial production of peroxide, oxidation of glutathione, and oxidation of mtDNA (*115*). However, a relationship between aging and apoptosis has not been established.

CONCLUDING REMARKS

According to the free radical theory of aging, proposed by Harman in the 1950s, oxygen-derived free radicals are responsible for the age-associated impairment at the cellular and tissue levels. This theory was developed by Miquel and Fleming, who suggested that mitochondria play a key role in cellular aging. Mitochondria, and especially their DNA (mtDNA), are major targets of free radical attack. At present, it is well established that mitochondrial deficits accumulate during aging because of oxidative damage. Thus, oxidative lesions to mtDNA accumulate with age in human and rodent tissues. In brain and liver, mitochondrial size increases, whereas mitochondrial membrane potential decreases with age.

Recently, we have shown that treatment with certain antioxidants, such as suphur-containing antioxidants, vitamins C and E, or the *Ginkgo biloba* extract EGb 761, protects against the age-associated oxidative damage to mitochondrial DNA and oxidation of mitochondrial glutathione. Moreover, the extract EGb 761 also prevents the changes in mitochondrial morphology and function associated with aging of the brain and liver. Thus, mitochondrial aging may be prevented by certain antioxidants. Furthermore, late-onset administration of certain antioxidants can also prevent the impairment in physiological performance, particularly in motor coordination, that occurs during aging.

On the other hand, mitochondria are key mediators of apoptosis because mitochondrial PT is a critical step in apoptosis. Indeed, opening of the mitochondrial PT pores releases apoptogenic factors from the intermembrane space. Mitochondria are at the same time the target and the source of ROS, which may induce apoptosis. Some common features of apoptotic cells and of cells from old animals include an increased mitochondrial production of peroxide, oxidation of glutathione, and oxidation of mtDNA. However, a relationship between aging and apoptosis remains to be established.

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