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The Fine Print:

Oxidative Stress and Aging

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Abbreviations:

CR: Caloric restriction.
CuZn SOD:Copper/znic SOD.
Ec SOD: Extracellular SOD.
Fe: Iron.
GSH: Glutathione.
HNE: 4-hydroxynonenal.
$HO^{\bullet}$: Hydroxyl radical.
MDA: Malondialdehyde.
Mt DNA: Mitochondrial DNA.
NOS: Nitric oxide synthase.
8-OHdG: 1-hydroxyldeoxyguanosine.
Q: Coenzyme Q.
SOD: Superoxide dismutase.
Cu: Copper.
DFO:Deferoxamine.
EPR:Electron paramagenetic resonace spin-trapping.
GPx: Glutathione peroxidase.
GSSG: Glutathione disulfide.
H_2O_2: Hydrogen peroxide.
HPLC: High performance liquid column.
Mn SOD: Manganese SOD.
$^{\bullet}NO$: Nitric oxide.
$O_2^{\bullet -}$: Superoxide anion.
$ONOO^-$: Peroxynitrite.
ROS: Reactive oxygen species.

Outline

Abstract

Aging is an inevitable process in every organism's life. Free radical theory of aging is one of the most widely accepted theories. In aerobic organisms, reactive oxygen species are by-products of their normal metabolisms. These reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radical, are highly reactive, they attack the important molecules in cell, damage the cell components. To survive under such threats, organisms have developed intricate defense mechanisms against free radicals. However, the antioxidant defenses are not strong enough to remove all ROS produced during physiological and pathological processes. Then oxidative damage occurs, if the damage cannot be repaired, it will accumulate with aging. The free radical theory of aging is one of the well-studied theories in aging. Numerous evidences in aging research support this theory. Caloric restriction has been shown to extend life span in a wide range of species and in rodents, and it also slows the progression of a variety of age-related diseases. Many early investigators used exogenous antioxidants in experimental diets to slow the biological deteriorations of aging caused by the oxidative damage from free radical reactions. This paper will review ROS generation, antioxidant defenses, oxidative stress responses, and these systems change with aging, as well as some therapeutic strategies based on free radical theory. A proposal to support the free radical theory of aging will also be presented.

Introduction

Reactive oxygen species (ROS) are continuously generated during normal respiring system, between 0.4 and 4% of all oxygen consumed is converted into the superoxide free radicals [1]. Free radicals have the capacity to oxidize proteins, lipids, DNA, and RNA [2,3]. Eukaryotes have evolved defenses against the free radical by-products of oxidative phosphorylation. The primary defense if the mitochondrial form of superoxide dismutase whose role is to detoxify superoxide ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2). The less active H_2O_2 can then either diffuse out of the mitochondrion and be converted into water by cytosolic catalase or, confine within mitochondrion and be converted into H_2O_2 by mitochondrial glutathione peroxidase (GPx) [4]. The ROS levels in the cell are dependent on the ROS production and antioxidant defense levels.

A variety of studies have suggested that oxidative stress is a major limiter of life span. The most compelling arguments come from genetic studies overexpression superoxide dismutase (*sod*) genes. The results have shown that these manipulations increased the mean and maximum life span of *Drosophila melanogaster* [5]. Caloric restriction (CR) has been demonstrated to extend the life span in mammals [6]. A couple groups show that oxidative damage with aging was modified by CR [7]. In this paper, the ROS generation, antioxidant defenses, oxidative damage and therapy strategies based on this theory to treat aging will be discussed.

Reactive oxygen species

Reactive oxygen species refer to molecules that contain oxygen and have higher reactivity than ground-state molecular oxygen. ROS include radicals such as superoxide ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$), nitric oxide ($^{\bullet}NO$), and non-radical species such as hydrogen peroxide

(H_2O_2), and peroxynitrite ($ONOO^-$) [8]. Some of these species, like $O_2^{\bullet -}$ and $HO^{\bullet}$, are extremely unstable, others, like H_2O_2, are freely diffusible and relatively long-lived.

ROS is primarily generated in mitochondria where the electron transport chain is. The electron transport chain is comprised of four complexes: complex I-IV [9]. The production of superoxide radicals occurs primarily at two discrete points, complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome *c* reductase). Under normal metabolic conditions, complex III is the main site of ROS production [10]. Electrons from complex I or II dehydrogenases are transferred to coenzyme Q (Q), also called ubiqunione. The resulting reduced form (QH_2) of coenzyme Q subsequently undergoes two sequential one-electron reductions (the Q cycle) using oxidized and reduced forms of cytochome *c* (Cyt *c*). The unstable intermediate in the Q cycle ($Q^{\bullet -}$) can lead to superoxide formation by transferring electrons directly to molecular oxygen (Fig 1). The superoxide once generated can be converted into hydrogen peroxide by superoxide dismutase, and H_2O_2 is metabolized by catalase and glutathione to regenerate water and oxygen. The generation of $O_2^{\bullet -}$ in this process is non-enzymatic and hence the higher the metabolism, the greater the production of ROS.

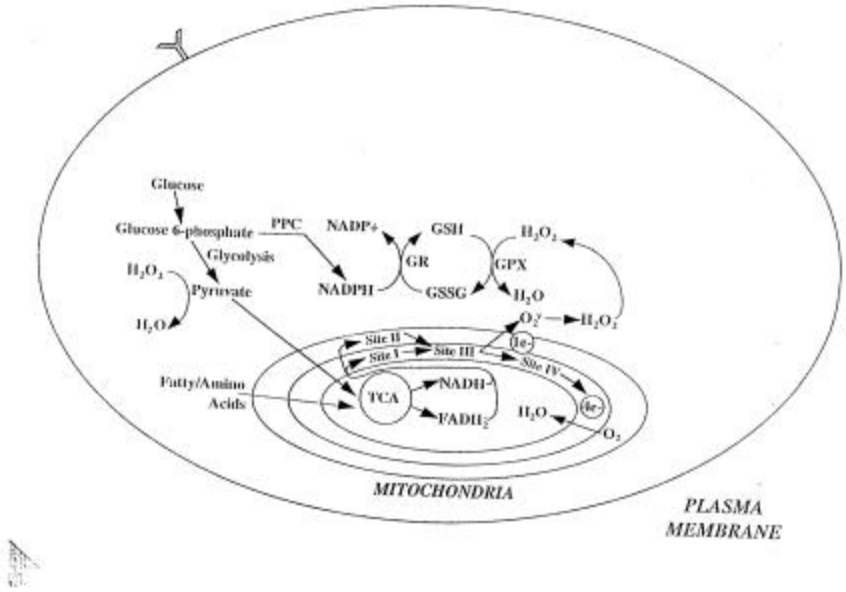

Figure 1: Scheme of a mitochondrion. Electrons are leaking from different locations. Adapted from [54].

Cytosolic enzyme systems are also contributed to ROS generation. The NADPH oxidases family, which was first discovered in the neutrophil, is a superoxide-generating system [11].

In the presence of transition metals such as Fe^{2+}, Cu^{2+}, H_2O_2 can generate hydroxyl radical though Fenton type reaction [13]. Iron is present in biological systems mostly as an essential part of proteins such as hemoglobin, ferrin, cytochromes, iron sulfyl (Fe-S) proteins, etc. Hydroxyl radical can rapidly react with any molecules next to it to generate other free radicals. In this case, a free radical chain reaction is set in motion until antioxidants come to detoxify and stop it.

Antioxidant defenses

Nature has evolved several enzymes and antioxidants that act in a combination fashion to help protect biological systems against oxidative damage. Their primary function is to lower the amount of oxygen free radicals or their precursors in the cell.

Superoxide dismutase acts to dismutate superoxide to form hydrogen peroxide and oxygen at a very high rate constant. Three forms of SOD exist in mammals: extracellular form (EcSOD), Mitochodrial form (manganese SOD), and cytosolic form (copper/zinc SOD)[15]. Catalase catalyzes to convert hydrogen peroxide to molecular oxygen and water; Glutathione peroxidase reduces hydroperoxide to alcohols and also reduces hydrogen peroxide to water; glutathione S-transferase that reduces hydroperxides to alcohols, and so forth. Figure 2 shows the schemes how these enzymes work [14].

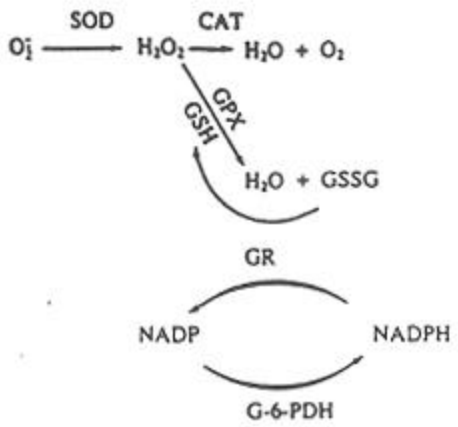

Figure 2: The antioxidant enzymes system in cells. Adapted from [14].

Biological antioxidants include water-soluble antioxidants, such as glutathione (reduced form: GSH, and glutathione disulfide: GSSG), ascorbic acid, and uric acid, and fat-soluble antioxidants, such as vitamin E, ubiquinones, and carotenoids. Glutathione is one of the most abundant reductants and acts as a thiol donor converting disulfides to thiols and as a cofactor for GPx and glutathione S-transferase. Vitamin E and ubiquiones exist mainly in the biomembranes and function as radical scavengers. Ascorbic acid is also a biological abundant reductant; it recycles vitamin E in scavenging lipid peroxidation. Uric acid and carotenoids are presumed to behave as singlet oxygen quenchers and radical scavengers [12].

Antioxidant systems are not always enough to scavenge all the free radicals; oxidative damage sometimes is inevitable *in vivo*. Oxidative damage repairing system is also thought to be necessary. Oxidized DNA may be repaired by endonuclease and glycosylase. Oxidized proteins may be removed by proteases. Oxidized lipids may be reduced by glutathione peroxidase or hydrolyzed by phospholipase [12].

Studies on antioxidant defenses changes with aging have not got consistent observations. It is species, strain, and tissue specific. In mice liver homogenates, catalase activity was

decreased [52]. SOD activity measured in homogenates of brain, heart, and kidney from C57BL/6Nina mice showed no clear trend [53].

Oxidative damage of macromolecules with aging

In reality, small amount of ROS escapes of the antioxidant defenses and repairing systems. Though this small amount of ROS is required for triggering or signaling events in the process of development or differentiation, it also causes oxidative damage. Many studies suggest that oxidative damage is accumulated during aging [16]. Perhaps the most crucial need with regard to understanding the role of ROS in aging is to obtain reliable quantitative data on the balance between ROS generation and antioxidant defenses in various tissues. Although it is relatively easy to measure antioxidants activities, it is extremely hard to obtain absolute values for the rate of free radical generation *in vivo*. Now it is generally accepted that some biomarkers are used to measure the oxidative damage, such as protein carbonylation for protein damage, 8-hydroxydeoxyguanosine (8-OhdG) for DNA damage [17].

Lipid

Many groups have found that lipid peroxidation increases with age [16]. It is reported that the amount of lipid hydroperoxide were higher in the mitochondria and microsomes taken from old rats compared to the levels found in young rats (Table 1) [55].

Age (Mons)	Mitochondria	Microsomes
6	11.31±0.38	1.47±0.33
12	1.57±0.10	2.81±0.15
24	1.98±0.29	2.42±0.29

Table 1: Membrane hydroperoxide (nmoles/mg Protein). Adapted from [55].

Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are products of lipid peroxidation. Yu *et al*. reported that both MDA and HNE increase in aged rat liver comparing to young rat liver [19]. And they further found that increases amount of MDA and HNE are responsible for loss of membrane fluidity with aging *in vitro* [19]. . It is also noticed that fatty acids compositions change with aging, the percentage of unsaturated fatty acids increase in aged rats. It is observed that 18:2 and 18:3 fatty acids were decreased and highly peroxidizable, unsaturated 20:4, 22:5, and 22:6 fatty acids were increased. [19].

Protein

Since proteins are major cellular structural and functional constituents, they also are targets of oxidative modification by free radicals [18]. Data from several laboratories supports the contention that the accumulation of oxidized protein occurs in a number of tissues during normal aging and that this damage is associated with behavioral and functional impairments. Metal-catalyzed oxidation of proteins introduces carbonyl groups at lysine, arginine, proline, or threonine in a site-specific manner while oxidative modification converts the side-chain of methionine, histidine, and tyrosine and forms cysteine disulfide bonds. *In vitro* oxidation of proteins is usually accompanied by structural modification, and resulting in physicochemical changes, such as fragmentation, aggregation, and increased susceptibility to proteolysis. Available data suggest that modification of many proteins is taken place *in vivo* [18]. Oxidation of proteins, specifically the histidine moieties, leads to increased carbonyl values, this increased carbonyl values are used as an index of protein oxidation to assess the age-related increase in oxidatively altered proteins.

DNA

Free radical-induced reactions with nucleic acid produce a range of modifications including adducts of base and sugar groups, single-and double-strand breaks, and cross-links to proteins [20]. Levels of oxidized nucleotide 8-hydroxy-deoxyguanosine (8-OH-dG), a biomaker of DNA damage, have been shown to accumulate with aging [21]. Perhaps because of its proximity to the main source of oxidant generation, or because of a limited DNA repair system, mitochondrial is generally considered to be even more sensitive than nuclear DNA to oxidative damage. MtDNA encodes most of the electron transport chain proteins. Because of mtDNA damage, these proteins may not be repaired. Consequently, these defects result in an increased generation of ROS and respiring deficits leading to death. In several tissues, including the central nerve system and muscle, levels of 8-OHdG in mtDNA exceed nuclear DNA (nDNA) 16-fold [21]. Several studies indicate that 8-OHdG most frequently base pairs correctly with cytosine, but also mispairs to adenine with 1% chance [22]. Therefore increased level of 8-OH-dG is correlated with increased genome instability, such as mutation and deletion, which is part of the reason for aging process and age-related diseases.

Oxidants in cellular signaling and senescence

Reactive oxygen species are not only metabolic by-products, they also serve as cellular signaling molecules. Under many environmental stimuli including cytokines, ultraviolet radiation, hyperthermia and even undergrowth factors generate high levels of ROS that can perturb the normal redox balance and shift cells into a state of oxidative stress. Survival or death of the affected cell is dependent on the ability of the cell to adapt to or resist the stress, and to repair or replace the damaged molecules.

A number of main stress signaling pathways are activated in response to oxidants injury, such as the nuclear factor (NF?B) signaling system, p53 activation, and the heat shock response, etc. Several of these pathways show diminished activity as a function of aging. In a variety of model systems and stress paradigms including oxidative tress, the magnitude of induction of heat-shock proteins, and the inducible (Hsp70) in particular, is attenuated with aging [24]. The heat shock protein family encompasses many chaperones involved in regulating the folding, transport and degradation of other cellular proteins. The relationship between aging and a decline in this stress response is unclear, but evidence indicates that elevating levels of Hsp70 enhances the survival of stressed cells and/or animals, while inhibiting this stress response reduces survival [24,25].

In contrast to the age-related attention in heat-shock protein induction in response to acute stress, recent studies using complementary DNA microarray analysis to examine age-associated changes in gene expression in mouse tissues provided evidence that basal expression of certain heat-shock proteins actually increases with aging [26]. Data from Kregel group also support this conclusion [24]. This elevation expression is interpreted to occur as a response to the age-associated accumulation of oxidatively damaged proteins. Basal DNA binding activities of NF?B and AP-1 have been shown to increase with age, which again has been suggested to reflect increased oxidative stress in aged cells and tissues [27]

From discussed above, the free radical theory of aging can be summarized in figure 3. According to this theory, a condition of oxidative stress ensues that well regulated and balanced redox reactions will become out of control under various of physiological and pathological conditions, and eventually leads to aging and diseases.

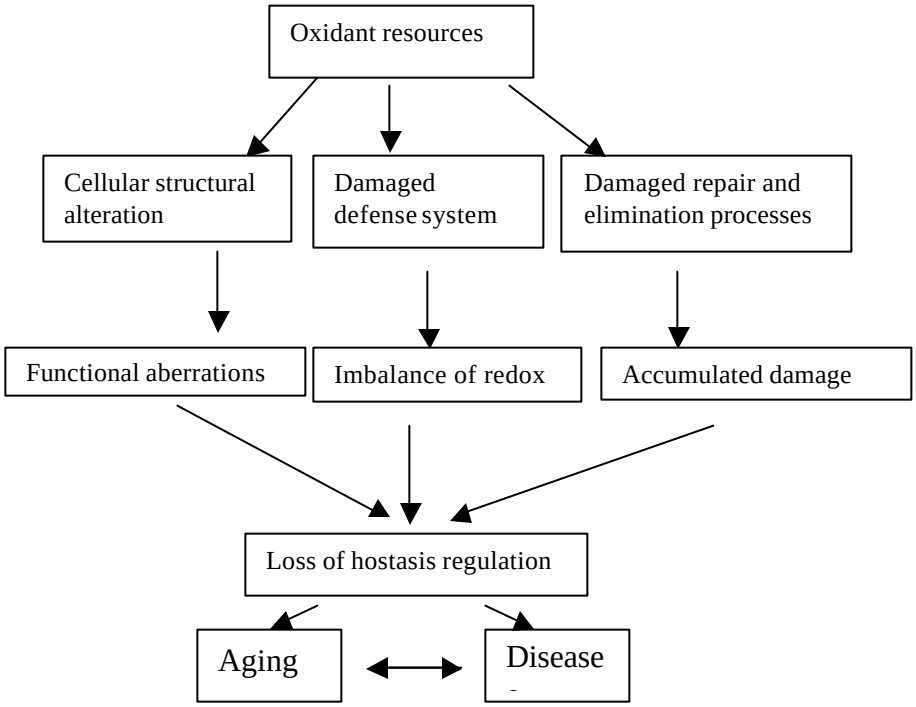

Figure 3: Schematic presentation of oxidative stress hypothesis shows various ROS and the altered processes that lead to aging and disease. Adapted from [56]

Therapeutic strategies against aging

There is tremendous public interest in the development of anti-aging therapies. If the free radical theory of aging is true, and the ability to resist or prevent oxidative stress is a key determinant of longevity, then it is likely that strategies aimed either at reducing the oxidative burden or improve host defense mechanisms involved in coping with the damage would have significant anti-aging effects.

Caloric restriction (CR)

Limiting food intake, or caloric restriction, has been shown to extend life span in a wide range of species and in rodents, and it also slows the progression of a variety of age-related diseases [28]. Based on some published studies that using rats and mice maintained under varying degree of severity of energy restriction revealed a significant negative correlation

between survival and energy intake [29]. It is clear that a dose-response relationship exists over a wide range of energy intake between the intensity of energy restriction and survival achieved. The shape of the survival curve is unaltered but moved to the right. CR not only extends the mean life span, but also increases the maximum life span of the organisms [29].

Amelioration of the rate of accrual of oxidative damage with age is considered to be the best mechanistic explanation to explain this dynamic and wide-ranging effect of CR on survival, age-related physiology and pathology [30].

Koizumi *et al.* were the first to show that extended survival in CR mice (approximately 40% restriction) was associated with 30% decrease in liver lipid peroxidation at 12 months of age and 13% at 24 months of age [31]. Yu group has studied the lipid composition of the mitochondria and microsomal membrane; they found that CR feeding induces a lower unsaturation/saturation ratio [32]. These changes are reverse of the membrane composition changes seen with age with fully fed animals, a decrease in 18:2 and 18:3 and increase in the highly peroxidizable, unsaturated 20:4, 22:5, and 22:6 fatty acids [32]. Increased membrane fluidity by CR is also reported [33].

Studies indicated that the age-related, steady state increase in carbonyl content of whole-tissues homogenates was significantly reduced by CR in mice [34]. But this decrease shows a tissues specific pattern: some decrease was observed in whole brain [35], while the mitochondria isolated from the hind limb skeletal muscle show no change [36].

The effect of CR on DNA damage is also explored. A detail study of nuclear 8-OHdG accumulation on Fischer 344 rat found that CR retarded the onset of increase in 8-OHdG concentration after 24 months of age, but not younger [37]. Because of its critical role in energy and ROS production, mitochondrial DNA is the most intensely studied. CR has been shown

retarded the age-related increase in mtDNA in the liver of Fischer 344 male rats [38]. Figure 3 shows the result.

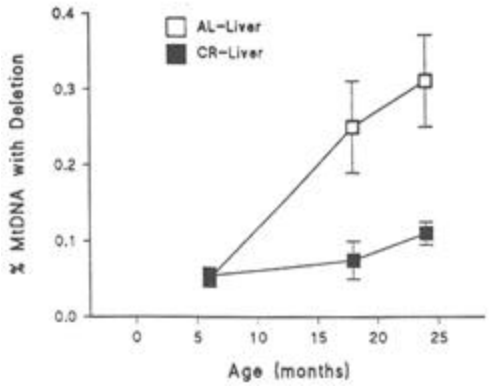

Figure 3: Effect of caloric restriction in Fischer 344 rats on the frequency of deleted mtDNA in liver. Adapted from [38]

Although CR is proved to be effective to extend life span in mammals, it is difficult to apply to human because of the ethical and practical difficulties. Better understanding of specific mechanisms responsible for the anti-aging effect of CR could lead to develop pharmacological agents that would be effective in slowing aging.

Antioxidant enzymes and antioxidants supplementation

Antioxidant enzymes and small molecular antioxidants have been used to decrease aging rate based on free radical theory of aging. Data from many groups are very encouraging. Based on the rational that SOD converting $O_2^{\bullet -}$ to H_2O_2, and catalase breaks down H_2O_2 into water and oxygen, Sohal *et al*. overexpressed both Cu-Zn SOD and catalase in *Drosophila melanogaster* [39]. This modulation increased both mean and maximum life span up to one third compared to the controls. Table 2 shows the antioxidant enzyme activities and mortality rates. To confirm that ROS are indeed a causal factor in aging, they also measured protein carbonyl content, physical performance, and metabolic potential. The age-related increase in carbonyl content was significantly slower in the SOD and catalase transgenic lines as compared t the control flies; a

higher percentage of transgenic flies retained the specified speed of walking (1 cm s^{-1}) compared to control group; and the average metabolic rate if the transgenic flies was similar to that of the control flies at younger ages but was higher than that of the controls at older ages [39].

Group	Strain	SOD activity (units)	CAT activity (units)	Mortality (days)			MRDT (days)
				median	90%	100%	
Control	Control	13.1±0.6	19.2±0.8	54.5	64	71	8.4
A	cat1.5 sod1.4	16.5±0.3	33.2±0.9	63	78	95	10.1
B	cat1.1 sod1.4	17.3±0.1	27.5±0.4	58	74	81	9.9
C	cat1.1 sod1.2	16.8±0.5	31.6±0.7	72.5	83	93	11.5

Table 2: Comparison SOD and catalase activities, and mortality pattern between transgenic and control groups in *Drosophila.* MRDT means mortality rate doubling time, calculated the slopes of the Gompertz plots. Adapted from [39].

Overexppression of Mn SOD only in *Drosophila* was performed in 1999. Unlikely to the Cu-Zn SOD and catalase group, the metabolic rate, level of physical activity, or the level of Cu-Zn SOD, catalase and GSH did not change. Moreover, the mean longevity of the transgenic flies was decreased by 4-5% [40]. Interestingly, expression of human SOD1 exclusively in *Drosophila* adult motor neurons leads to a 40% extension in life span [51].

Small molecular antioxidants such as vitamin E, C used to modulate aging process [56]. Although some success has been achieved in improve the disorders caused by oxidative damage, these strategies have little or no effect in enhancing longevity. Furthermore, success application of this approach will require much more understanding the mechanisms and pharmacological properties of these agents. For example, vitamin C and E can act both as antioxidants and proxidants dependent on the conditions [42]. The development of SOD and catalase mimetics has offered an alternative approach with some promise. Use of such compounds in *C. elegans* has shown extension of longevity [43].

Proposal of future study

Though aging is likely to be a multifactorial process, significant evidence suggests that longevity is implicated the generation of ROS and the corresponding response to oxidative stress. Much of the evidence was correlative, not casual. Increasing evidence will make this theory more convincing. Based on the evidence that hydroxyl radical produced by transition metal catalyzed Fenton reaction is an important free radical *in vivo*, I propose that administration of metal chelators, which decrease the free transition metal concentration *in vivo*, can decrease the oxidative damage, then decreases the rate of aging, and eventually extends the life span of organisms.

Hypothesis

Reactive oxygen species is a causal factor in aging. Administration of metal chelators, such as Deferoxamine (DFO) and desferal, will decrease the production of highly harmful hydroxyl radical, and decrease oxidative damage, eventually extend the life span of *C. elegans.*

Rational

The electrons leaked from the mitochondrial complexes I and III come out as $O_2^{\bullet -}$, and Mn SOD converts $O_2^{\bullet -}$ into H_2O_2, H_2O_2 can be detoxified into alcohol by GPx in mitochondria. In the presence of transition metals, such as Fe^{3+}, Cu^{2+}, H_2O_2 will react with Fe^{2+}, which is produced from Fe^{3+} reacts with $O_2^{\cdot -}$, to produce extremely reactive hydroxyl radical [13] (reaction 1,2)

$$O_2^{\cdot -} + Fe\ (III)\ ?\ \ O_2 + Fe\ (II) \qquad (1)$$

$$Fe\ (II) + H_2O_2\ ?\ \ OH^{\bullet} + OH^{-} + Fe\ (III) \qquad k_2 = 761\ M^{-1}S^{-1} \qquad (2)$$

Because mtDNA, lipid and protein are so close to $OH^{\bullet}$, oxidative damage is inevitable. Oxidatively damaged DNA, lipid, and proteins, if not repaired, the DNA will encode some modulated proteins, which will decrease the efficiency of the electron transport chain, and leaks more electrons; lipid become more permeable, and leak more ROS into cytosol; proteins become less functional, so the complexes leak more electron, the enzymes will detoxify less ROS. Cuajungco *et al.* found that there was abnormal metal accumulation in aged Alzheimer's disease [44]. If ROS are indeed a causal factor in aging, decease ROS generation through administration metal chelators should reduce oxidative stress, decrease the rate of aging, and extend life span.

One group in Harvard medical school has considered to develop the drugs target abnormal metal accumulation and its adverse consequences [44]. Other group found that iron chelators prevents oxidative stress-induced apoptosis by increasing DNA binding of hypoxia-inducible factor-1, and increased expression of glycolytic enzymes [45]. Metal chelators, such as desferal, N-acetyl penicillamine, found to suppress the erythrocytic oxidative damage generated during *Plasmodium berghei* infection [46]. Metal chelators decreased the levels of $O_2^{\bullet-}$, lipid peroxidation in infected animals [46]. Deferoxamine (DFO), an iron chelator, has been reported to slow progression of Alzheimer's disease in clinic [48].

SOD and catalase mimetics have been proved to extend the life span of *C.elegans* [43]. Since these mimetics can remove $O_2^{\bullet-}$ and HO, and DFO/desferal can prevent hydroxyl radical formation, combination of these two therapies may have some addition effects.

Specific aim 1: Determining if the life span of *C. elegans* can be extended by DFO/desferal supplementation; and if this modulation changes the oxidative damage in the worms.

Measure the life spans

Treat one group of worms (n = 10 for each group) in medium with different of concentrations of DFO or desferal; and maintain the control group in medium without the metal chelators. The adult worms are transferred each day to new medium during the worm's reproductive period and every 3 days thereafter. M*ev-1* gene mutant worm, which lacks of cytochrome b subunit of succinate dehydrogenase in the electron transport chain, has a phenotype of shorted life span [43]. M*ev-1* gene mutant worms are also submitted to with or without DFO/desferal supplementation. Measure the individual life span of every worm in days. Because genetic manipulation of the life span is generally associated with effects on life history traits such as altered growth rate [48], so the body weights of each worm will be measured at day 6, day12, day18.

Detect the hydroxyl radical

Feed the control and DFO/desferal group as above, harvest the tissues of worms at 6 days, 12 days, and 18days.

Detect the hydroxyl radical in control and experiment groups using Electron paramagnetic resonance spin-trapping (EPR).

Measure the oxidative damage markers

1) Determination of lipid peroxidation

The determination of total lipid hydroperoxides will be done with the Cayman LPO Assay kit, the unstable hydroperoxides react with ferrous ions; the resulting ferric ions are detected using thiocyanate as the chromogen [49].

2) Determination of protein carbonyl content

Protein carbonyl content is measured in homegenates from each group. The 2,4-dinitrophenyl hydrazine (DNPH) procedure will be used here [50].

3) Determination of 8-OHdG content of nuclear and mitochondrial DNA

Mitochondria and nucleus are separated by differential centrifugation. Nuclear and mitochondrial DNA is extracted with phenol and precipitated with cold ethanol from nuclear and mitochondrion. The isolated DNA is treated with RNase to remove RNA contamination. The amount of 8-OHdG in DNA is estimated by HPLC [50].

Specific aim 2: Determination the effect of combining of DFO/desferal with SOD and catalase mimetics.

Experiment design is the same as specific aim 1. Analyzing the results by comparing the results from specific aim 1 and table 1.

Conclusion

As described above, oxidative stress is an important factor in aging. The ROS produced continuously and physiologically *in vivo*, attacks the important macromolecules and cause oxidative damage, which eventually leads to aging. The evidence which supports the free radical theory of aging is increasing. However, most of the evidence is correlative, the details of the basic underlying mechanisms are still not fully elucidated and remain controversial. Recently the identification of longevity influencing genes significantly strengthens the mechanistic connection between oxidants, stress, and aging. Moreover, the relationship between the telomeric shortening and oxidative stress also has been studied. But how these factors work together to influence the aging process is still unclear. Answers to this question will undoubtedly help determine whether ROS plays merely a correlative factor with aging, or instead, is a central regulator of aging.

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