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Evaluation of Role of Oxidative Stress on Aging in *Caenorhabditis elegans*: A Brief Review

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Abstract: Recently the relationship between oxidative stress and aging has been brought into question. It has been suggested that while oxidative events may play a role in the progression of age-related pathologies, it is not relevant to aging processes not involving specific diseases associated with senescence. The evidence in support of this concept is largely based on studies with the roundworm, *Caenorhabditis elegans* (*C. elegans*) that has been extensively used as a model system to study aging. This commentary evaluates data derived from *C. elegans* and documents that the preponderance of evidence from this species supports the role of pro-oxidant events as being a significant contributor to normal aging. Possible reasons for some anomalous findings conflicting with this concept, are discussed.

Keywords: Antioxidants, aging, *Caenorhabditis elegans*, lifespan, oxidative stress, superoxide.

INTRODUCTION

The length of lifespan of a particular normal cell of any organism is predetermined. Similarly, the length of lifespan of all organisms is pre-determined by their genetic makeup and their external and internal environments and diet-related factors specific to an organism. Therefore, the length of lifespan can be increased or decreased by manipulating the environment, diet and genetic factors only by small extent. The length of lifespan of the organisms can also be impacted by differential rates of senescence of cells and organs that ultimately lead to the death of the organisms. The differential rates of cellular senescence are influenced by several confounding factors, such as external and internal environments, diet and genetic factors. Because of these confounding factors that can impact rates of progression of degenerative changes in the organs, it is almost impossible to study aging in the absence of organ pathology. Based on numerous studies on aging in vertebrates and invertebrates, a recent informative review has suggested that oxidative stress theory of aging can only be applied to conditions in which age associated pathologies are included [1]. Furthermore, it was suggested that in environment with minimal stress, oxidative damage plays little role in aging. This suggestion can be argued on the fact that little oxidative damage may take longer time to deregulate protective transcription factors, adaptive responses to stressors, and repair mechanisms, and thereby extending the lifespan of the organisms more than that produced by higher oxidative damage which can deregulate above biological functions in shorter time.

Using vertebrate and invertebrate models, some major biochemical and genetic factors that are associated with

aging processes have been identified. They include increased oxidative stress and chronic inflammation, decreased adaptive response to stressors, post-translational protein modifications, mitochondrial dysfunction, decreased of proteasome and lysosomal-mediated proteolytic activity, shortening of telomeres and transcriptional deregulation. Among these, the theory of oxidative stress is most extensively investigated in various experimental models, using pharmacological agents, antioxidants, anti-inflammatory agents and deletion of one or more antioxidant enzymes as well as of mitochondrial complexes. Depending upon the experimental models, experimental designs, and substrate used to assay oxidative stress and criteria of oxidative stress, the role of oxidative stress in aging has been substantiated or questioned. We hypothesized that increased oxidative stress may be one of the primary early events that causes chronic inflammation, transcriptional deregulation, post-translational protein modifications, mitochondrial dysfunction, decreased of proteasome and lysosomal-mediated proteolytic activity and shortening of telomeres.

Invertebrate models, such as *Caenorhabditis elegans* (*C. elegans*) has been extensively used to evaluate the role of oxidative stress in aging primarily due to shorter lifespan of about 3 days and ease of genetic manipulation. This review analyzes recent published studies on *C. elegans* on the role of oxidative stress in determining the length of lifespan by generating mutants that show suppression of mitochondrial function or lack of superoxide dismutase (SOD).

Studies on Mutants *C. elegans*

Caenorhabditis elegans (*C. elegans*) has been extensively used to investigate the role of oxidative stress in aging by measuring the length of lifespan. Mitochondria are considered the major sites for the production Reactive oxygen species (ROS), although ROS are also produced outside the

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mitochondria. In order to demonstrate the impact of oxidative stress, several mutants of *C. elegans* were generated. They include mutations in four clock genes (clk-1, clk-2, clk-3 and clk-4), mutation in the iron sulfur protein (isp-1) of mitochondrial complex III, mutation in the gene NUO-6 and mutation in the gene daf-2. The effects of mutations on oxidative stress and lifespan are summarized in Table 1.

The clk-1 gene encodes an enzyme that is necessary for the biosynthesis of ubiquinone that is required by the mitochondria to generate energy. Mutation in the clk-1 gene increases the life span by slowing down mitochondrial activity due to reduced availability of ubiquinone. This slowing of the electron transport chain would reduce oxidative stress. The role of reduced oxidative stress in extending the lifespan is further supported by the fact that overexpression of clk-1 gene in wild-type *C. elegans* increased mitochondrial activity and shortened the lifespan [2]. A mutation in the iron sulfur protein (isp-1) of mitochondrial complex III causes low oxygen consumption, reduced oxidative stress and increased lifespan [3]. Mutation in the daf-2 gene which codes for a member of insulin receptor family increased lifespan and enhanced resistance to oxidative stress [4]. In this daf-2 mutant, expression of the SOD-3 gene, which encodes mitochondrial Mn-superoxide dismutase, was much higher than in the wild type. This implies that the increased levels of SOD-3 in the daf-2 mutant reduced oxidative stress and thereby increased lifespan. Mutation in the gene NUO-6 which encodes complex I of mitochondria increases life span of *C. elegans* by decreasing the mitochondrial function [5]. Mutation in the age-1 increased lifespan by two folds. This mutant worm had increased catalase and Cu/Zn SOD activities which may account for the increased resistance to the paraquat, a superoxide generating chemical [6]. The mutants *C. elegans* support the view that the levels of oxidative stress is one of the important determinant factors in determining the length of lifespan

Studies on *C. elegans* with Elevated Levels of Superoxide

Superoxide anions are produced enzymatically outside the mitochondria by different oxidases and non-enzymatically inside the mitochondria [7]. SOD detoxifies superoxide to hydrogen peroxide (H₂O₂), which is converted to water and oxygen by catalase. There are five superoxide dismutase (SOD) isoforms SOD-1, SOD-2, SOD-3, SOD-4 and SOD-5 in *C. elegans*. However, in most organisms there are only 3 SODs. SOD-1 is present in the cytoplasm and represents the majority of SOD activity, whereas SOD-2 and SOD-3 are present in mitochondria. Increased levels of superoxide were observed in SOD deleted wild type worms or in ISP-1 and NOU-6 mutants. The effect of deletion of SOD on *C. elegans* lifespan is shown in Table 2.

The impact of SOD deletion on the lifespan appears to be contradictory, depending upon the *C. elegans* model used. For example, SOD2 deletion markedly increased the lifespan of mutant clk-1 worms, but it decreased the lifespan of mutant isp-1 worms [8]. In addition, deletion of individual SOD genes from wild type *C. elegans* did not decrease the lifespan of these worms. This is in sharp contrast to other model, such as yeast, flies, and mice in which deletion of cytoplasmic or mitochondrial SOD caused decreased in the lifespan [1].

In another study, it was demonstrated that the levels of superoxide were elevated in nou-6 mutant and isp-1 mutant and they lived longer than the wild type, however, the oxidative stress was low and overall levels of ROS did not change [5]. From these results, it was concluded that elevation of superoxide is sufficient to increase the lifespan of these mutant worms. Based on these results, the role of oxidative stress in aging was questioned. It should be pointed out that if superoxide is precursor of ROS, the levels of ROS and oxidative stress should have been increased. This was not observed in the above study, suggesting that reduced oxidative stress possibly due to adaptive response by other anti-

Table 1. Consequences of Mutations in *C. elegans* on Oxidative Stress and Lifespan

Mutation in Gene	Effects	Oxidative Stress	Lifespan
Clk-1	Reduced mitochondrial activity	Reduced	Increased
Isp-1	“ “ “	“	“
NUO-6	“ “ “	“	“
Daf-2	Increased Mn-SOD	“	“
Age-1	Increased Cu/Zn SOD	“	“

Table 2. Effect of Gene Deletion of SOD on the Lifespan of *C. elegans*

Worm Type	SOD Deletion	lifespan
Mutant clk-1	SOD-2	Increased
Mutant isp-1	SOD-2	Decreased
Wild type	All SODs individually	No effect
Wild type	All SODs together	No effect

N. B. In contrast to *C. elegans*, deletion of SOD in yeast, *Drosophila melanogaster* or mice decreases lifespan.

oxidant enzymes, such as catalase and glutathione peroxidase and improved repair mechanisms was responsible for the extension of lifespan of these mutant worms. In the same article, it was observed that addition of N-acetylcysteine (NAC) and vitamin C individually abolished the effect of superoxide on life extension and other associated changes in *nou-6* and *isp-1* mutants. Vitamin C in water is rapidly oxidized and can act as a pro-oxidant. The antioxidant effect of NAC is mediated *via* glutathione, which in the presence of the high superoxide environment of mutant worms can be oxidized and then act as a pro-oxidant. Therefore, observed abolition of the effect of superoxide on life extension could be related to pro-oxidant effects of NAC and vitamin C.

In order to assess the role of SOD on life extension further, a model of *C. elegans* (SOD⁻ 12345) was developed in which all five SODs were deleted [8]. The results showed that SOD 12345 worms were viable and exhibited a normal lifespan similar to that of wild-type despite increased sensitivity to multiple stressors. However, these SOD lacking worms showed reduced fertility, slow development, slower defecation cycle and decreased movement (thrashing in liquid). From these results, it was concluded that SOD is dispensable for normal lifespan of *C. elegans*. This is in sharp contrast to mammals in which SOD is considered indispensable for survival. Thus, the results obtained on some genetic models of *C. elegans* cannot readily be extrapolated to the genetic models of mammals.

If SOD is dispensable for the survival and lifespan of *C. elegans* as reported recently [8], overexpression of SOD should have no impact on the lifespan of these worms. On the contrary, it was reported that overexpression of the major cytosolic Cu/Zn-SOD (SOD-1) increased lifespan of wild type worms which was not related to reduced lipid oxidation or glycation. As a matter of fact, the levels of protein oxidation were increased in these worms. The life extension effect

of overexpression of SOD-1 was due to activation of longevity-promoting transcriptional factors, such as DAF-16/FoxO, heat shock factor-1 (HSF-1) protein [9]. Similarly, overexpression of mitochondrial Mn-SOD (SOD-2) also increased lifespan of the worms, which was dependent on *daf-16*. It was suggested that overexpression of SOD-1 may trigger a *daf-16* and HSF-1-dependent stress response that extends lifespan and not by removing superoxide. It is interesting to note that overexpression of SOD causes increased oxidation of protein and no change in lipid peroxidation. This would imply that proteins are more susceptible to oxidative damage than membrane lipids. It is possible that overexpression of SOD causes translocation of this antioxidant enzyme from the cytoplasm to the membrane where it protects lipid against oxidative damage in *C. elegans*. Over-expression of SOD can influence life extension in at least two ways as presented in (Fig. 1).

Studies on *C. elegans* with Antioxidant Supplementation

It has been difficult to deliver single antioxidants orally in *C. elegans*. Using a newly devised technique for an oral administration, it was demonstrated that supplementation with lipid soluble antioxidants tocotrienol, astaxanthin or gamma-tocopherol prolonged the lifespan of *C. elegans* [10]. In contrast, the same study revealed that adding these antioxidants to the growth medium or the plate (older conventional delivery methods) did not enhance the lifespan. These results suggest that adding antioxidant directly to the media may no yield consistent results in extending the lifespan. Treatment of worms during pre-reproductive and young adult stages with astaxanthin, a carotenoid present in marine animals and sea weeds, significantly extended the lifespan; this treatment was not effective in worms lacking *daf-16* [11]. The results also showed that astaxanthin treatment of wild-type worms increased expression of SOD and catalase

Fig. (1). Means by which overexpression of superoxide dismutase can influence lifespan.

in two weeks after hatching and daf-16 protein was translocated to the nucleus. It was proposed that the effect of astaxanthin on extension of lifespan was in part mediated *via* an Ins/Igf-1 signaling pathway. Treatment of *C. elegans* with cypermethrin (CYP) increased ROS and H₂O₂. Elevation of peptide carbonylation suggested free radical damage to proteins, and decreased lifespan [12]. Treatment with α -tocopherol prevented CYP-induced increase in oxidative stress and decline in lifespan [12]. Supplementation of *C. elegans* media with a mixture of polyphenols derived from blueberries increased lifespan and age-related declines in *C. elegans* [13]. This treatment also increased survival during acute heat stress. The blueberry extract used contained 3 major fractions all of which exhibited antioxidant activity, but only the fraction containing proanthocyanidins enhanced lifespan and thermotolerance. Treatment with crude *Ginkgo biloba* (*G. biloba*) extract (EGb 761) increased the lifespan of *C. elegans*. However, purified ingredients of *G. biloba* especially flavonoids and tamarixetin were most effective in extending lifespan [14]. *G. biloba* extract also increased resistance to oxidative stress and thermal stress in wild-type and premature aging *C. elegans* mutant (mev-1) [14]. Supplementation of *C. elegans* incubation media with a strain of lactic acid bacteria *Lactobacillus rhamnosus* CNCM-3690, which exhibits antioxidant activity, increased lifespan and protected against oxidative stress. This strain of lactic acid producing bacteria also reduced inflammation [15]. These studies suggest that increased levels of antioxidants extend the lifespan by reducing oxidative stress and possibly by other mechanisms, such as preventing deregulation of protective transcriptional factors. In another study, it was reported that superoxide generators paraquat and plumbagin reduced the length of lifespan [16]. This is consistent with oxidative theory of aging. It was further reported that treatment that treatment with synthetic SOD mimetics EUK-8 or EUK-134, increased the activities of SOD, but not the levels of SOD proteins. Treatment with EUK-8 or EUK-134 in combination with a superoxide generator increased the lifespan of wild type worms; however, in the absence of a superoxide generator, it failed to increase the lifespan. It is possible that superoxide generator induces adaptive responses led by catalase and glutathione peroxidase and other longevity factors such as transcriptional regulators of anti-stress proteins, and repair mechanisms. These adaptive responses help SOD mimetic in extending the lifespan. However, in the absence of these adaptive responses produced by treatment with a superoxide generator, SOD mimetic is ineffective in extending the lifespan.

Studies on *C. elegans* with Non-steroidal Anti-inflammatory Agents

The role of chronic inflammation on the lifespan has not been adequately investigated in *C. elegans*. In order to assess the role of chronic inflammation on the lifespan, anti-inflammatory agents have been used. Treatment with aspirin and salicylate treatment increased lifespan and delayed age-related declines, such as learning behavior (isothermal tracking), motor activity, thermal tolerance, osmotic resistance, brood size and intracellular protein aggregation in *C. elegans* by reducing ROS and increasing expressions of antioxidants genes encoding for SOD (especially SOD-3), catalase and

glutathione peroxidase [17]. Aspirin and salicylate treatment also reduced intracellular protein aggregation that is associated with increased aging. Aspirin treatment did not extend lifespan but improved resistance to stressors in worms lacking daf-16. Supplementation with a potent specific inhibitor of cyclooxygenase-2 (COX-2) celecoxib increased lifespan and age-related decline in *C. elegans* [18]. This effect of celecoxib was not related to its inhibitory effect on COX-2 activity because an analog of celecoxib, which lacks the COX-2 inhibitory effect, also enhanced lifespan of *C. elegans* [18]. The life-extending effect of celecoxib appears to be mediated *via* insulin/Igf-1 signaling pathways. Thus the effect of celecoxib on *C. elegans* life span resembles the consequence of SOD-3 over expression in the daf-2 mutant.

Lifespan can be extended by multiple pathways that include reduced oxidative stress and activation of longevity-promoting transcriptional factors, such as Daf-16 and heat shock factor-1 protein. Oxidative stress can be reduced by multiple pathways which include reduced activity of mitochondria by inhibiting activities of complexes, increased transcriptional factor Nrf2 which regulate antioxidant enzymes and elevated antioxidant levels which directly scavenge free radicals as well as enhance antioxidant enzymes levels. It is now established that low levels of superoxide is required to activate and translocate Nrf2 from the cytoplasm to the nucleus which reduces oxidative stress by up-regulating antioxidant enzymes. In *C. elegans*, the presence of increased levels of superoxide by deleting SOD-2 decreases the oxidative stress and extends the lifespan. It is possible that increased levels of superoxide triggers activation of Nrf2 that decreases oxidative stress by up-regulating other antioxidant enzymes. The role of Nrf2 in regulating antioxidant enzymes has not been evaluated. Although deletion of all 5 SODs had no impact on the lifespan of *C. elegans*, the worms lacking all SODs showed reduced fertility, slow development, slower defecation cycle and decreased movement [8]. These data suggest that adaptive responses by other antioxidant enzymes and transcriptional factors may have prevented reduction in lifespan. These data also suggest that all SODs are needed for normal growth and healthy life of worms

CONCLUSION

The maintenance of oxidation and reduction processes in a steady state is essential for maintaining normal function of the cells. Imbalances in the favor of oxidation causes increased oxidative stress that increases with the advancing age. Imbalances in the favor of oxidation may be genetically determined; they can be accelerated by environmental and dietary factors, which can increase the risk of developing age-related pathologies. The rate of aging processes is highly complex and is influenced by interaction between genetics, and environmental and dietary factors that can induced increased oxidative stress, mitochondrial dysfunction and transcriptional deregulation. The oxidative theory of aging has been most extensively studied. This theory was tested on *C. elegans* because of their very short lifespan and ease of genetically manipulation. The results of these studies supported as well as refuted the oxidative theory of aging, depending upon the mutants used. Most studies supported the view that increased oxidative damage can reduce the length of

lifespan. The aging process cannot be investigated in the total absence of oxidative stress. It is evident in mammals that increased chronic oxidative stress accelerates the rate of age-related decline in physiological and behavioral functioning, and reduces the length of lifespan. Therefore, the minimization of oxidative stress remains one of the most rational strategies for decreasing the rate of age-related decline in organ function and for promoting optimal aging.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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