Oxidative stress and aging – the use of superoxide dismutase/catalase mimetics to extend lifespan

J.N. Sampayo, M.S. Gill and G.J. Lithgow

Buck Institute, 8001 Redwood Boulevard, Novato, CA 94945, U.S.A.

Abstract

To date, more than 40 genes have been identified in the nematode Caenorhabditis elegans, which, when mutated, lead to an increase in lifespan. Of those tested, all confer an increased resistance to oxidative stress. In addition, the lifespan of C. elegans can also be extended by the administration of synthetic superoxide dismutase/catalase mimetics. These compounds also appear to confer resistance to oxidative damage, since they protect against paraquat treatment. The protective effects of these compounds are apparent with treatment during either development or adulthood. These findings have demonstrated that pharmacological intervention in the aging process is possible and that these compounds can provide important information about the underlying mechanisms. To date, such interventions have targeted known processes rather than screening compound libraries because of the limitations of assessing lifespan in nematodes. However, we have recently developed a microplate-based assay that allows for a rapid and objective score of nematode survival at rates many times higher than previously possible. This system now provides the opportunity to perform high-throughput screens for compounds that affect nematode survival in the face of acute oxidative stress and will facilitate the identification of novel drugs that extend nematode lifespan.

In the mid-1950s, Denham Harman proposed the ‘free radical theory of aging’, which postulated that the accumulation of damage caused by free radicals was the underlying mechanism by which organisms age [1,2]. The theory has since been transformed into a more general premise which highlights the importance of ROS (reactive oxygen species) in the aging process [3,4]. Furthermore, there is increasing evidence to implicate the accumulation of macromolecular damage caused by ROS in the etiology of a number of age-related disease states [5,6]. Despite the fact that the free radical theory of aging is generally accepted by most biological gerontologists as the most likely mechanistic cause of aging, it is based on very little direct experimental evidence [5].

ROS are a by-product of normal metabolism, and are produced during aerobic respiration when electrons are transferred along the respiratory chain to generate ATP. In a perfect system, oxygen would be the final electron acceptor, yielding water. However, the system is not perfect, and ROS are produced as a result of inappropriate electron donation, usually involving ubiquinone at Complex III [6]. In order to prevent oxidative damage to cellular components from occurring, a number of protective enzymes have evolved. SODs (superoxide dismutases) remove superoxide anions (O$_2^{-}$) by catalysing their conversion into hydrogen peroxide (H$_2$O$_2$), which in turn is broken down by catalase to yield oxygen and water. Other antioxidant enzymes are involved in maintaining the redox status of glutathione. Glutathione peroxidase removes H$_2$O$_2$ by using it to oxidize GSH to GSSG, while glutathione reductase regenerates GSH from GSSG, with NADPH as a source of reducing power. Observations in a number of species show that oxidative damage accumulates with age in a wide variety of tissues [7], and that the efficiency of cellular antioxidant defences decline with age [8,9], lending strong support to the free radical theory of aging.

Studies in the nematode roundworm Caenorhabditis elegans provide some of the best correlative evidence that ROS play a role in lifespan determination [10–14]. C. elegans is an important model organism in which to study the basic biology of aging, due to the identification of single gene mutations that dramatically extend lifespan under laboratory conditions (Age genes). To date, over 40 Age genes have been characterized that have effects on a wide variety of processes [15]. Of the different classes of genes that have been identified, those that affect an insulin-like signalling pathway and mitochondrial function are of particular interest with respect to the free radical theory of aging (Table 1).

One of the most well characterized groups of Age genes are those involved in an insulin/IGF-I (insulin-like growth factor-1)-like signalling pathway [16]. The products of these genes include DAF-2 (an insulin/IGF-I-like receptor) and AGE-1 (a p110 catalytic subunit of phosphoinositide 3-kinase). The lifespan extension conferred by both of these mutations is dependent on the action of a downstream target of the pathway, DAF-16, a member of the forkhead transcription factor family. A common characteristic of all Age mutants is resistance not only to oxidative stress, but also to a variety of other stressors. Moreover, both age-1 and daf-2 mutants have been shown to have elevated levels of the ROS detoxifying enzymes, SOD and catalase, in mid to late life [10,11]. Nematodes carrying the age-1 mutation also exhibit

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Key words: Age genes, aging, Caenorhabditis elegans, lifespan, oxidative stress, reactive oxygen species.

Abbreviations used: IGF, insulin-like growth factor; ROS, reactive oxygen species; SCA, superoxide dismutase/catalase mimetic; SOD, superoxide dismutase.

1To whom correspondence should be addressed (e-mail glithgow@buckinstitute.org).
A subset of nematode Age genes that are related to oxidative stress resistance

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<tr>
<th>Gene</th>
<th>Function</th>
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<tbody>
<tr>
<td>recessive mutations</td>
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<tr>
<td>age-1</td>
<td>PI 3-kinase (catalytic subunit p110)</td>
</tr>
<tr>
<td>clk-1</td>
<td>Strong similarity to the human ubiquinone biosynthesis protein COQ7</td>
</tr>
<tr>
<td>daf-2</td>
<td>Insulin-like/IGF-1 tyrosine kinase receptor</td>
</tr>
<tr>
<td>isp-1</td>
<td>Iron-sulphur protein of mitochondrial Complex III</td>
</tr>
<tr>
<td>lrs-2</td>
<td>Mitochondrial leucyl-tRNA synthetase</td>
</tr>
<tr>
<td>pdk-1</td>
<td>Serine/threonine kinase</td>
</tr>
<tr>
<td>unc-31</td>
<td>Homologue of vertebrate CAPS, a calcium-binding protein</td>
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<tr>
<td>unc-64</td>
<td>Syntaxin homologue</td>
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<tr>
<td>RNAi</td>
<td></td>
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<tr>
<td>irs-18</td>
<td>Insulin ligand predicted to bind DAF-2</td>
</tr>
<tr>
<td>overexpression</td>
<td></td>
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<tr>
<td>tkr-1</td>
<td>Tyrosine kinase receptor (now called old-1)</td>
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<tr>
<td>sir-2.1</td>
<td>Similarity to NAD+-dependent histone deacetylases</td>
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<tr>
<td>ins-1</td>
<td>Insulin-like protein</td>
</tr>
<tr>
<td>ins-18</td>
<td>Human insulin</td>
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<tr>
<td>Hsp70F</td>
<td>Possible heat-shock protein</td>
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<tr>
<td>Hsp16</td>
<td>Heat-shock protein</td>
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increased accumulation of small heat-shock proteins (HSP16) following heat shock [17], and there is some evidence that daf-2 mutants may have elevated hsp16 mRNA levels under non-heat-shock conditions [18]. Conversely, mutations in daf-16 render the worm sensitive to oxidative stress and heat shock, and abolish the increased stress resistance when combined with mutations in daf-2 or age-1 [17–20]. The importance of this insulin-signalling pathway in lifespan extension and oxidative stress resistance is not restricted to the nematode. Mutation of the Drosophila insulin receptor (InsR; Ins receptor mutation) and partial knockout of the mouse IGF-1 receptor also result in an extension of lifespan as well as an increase in resistance to paraquat-induced oxidative stress [21,22]. Further insight into the role of oxidative stress in aging in C. elegans has been gained by the analysis of mutants with altered mitochondrial function. Some of these mutants exhibit an extension in lifespan (e.g. clk-1 [23]), while others render the nematode short lived. One such mutation is mev-1 (succinate–CoQ oxidoreductase in the mitochondrial electron transport chain), isolated due to its hypersensitivity to methyl viologen (paraquat). Worms harbouring a mev-1 mutation are sensitive to oxidative stress and exhibit accelerated aging. They have additional phenotypes such as mitochondrial ultrastructural abnormalities, lowered mitochondrial membrane potential and increased rates of apoptosis during development [24], which provide further support for the free radical theory of aging.

A prediction of the free radical theory of aging is that damage associated with ROS, and thus aging itself, could be ameliorated by interventions that enhance resistance to oxidative stress. Transgenic overexpression of antioxidant enzyme genes has been shown to extend lifespan in Drosophila [25,26], and while this genetic approach is of importance in aging research, a range of pharmacological compounds with anti-aging properties would be of enormous value in the study of normal aging, functional decline and age-related disease across species. This latter approach has been evaluated in C. elegans through the administration of antioxidant compounds with the aim of reducing oxidative stress and thereby extending lifespan.

Initial studies using vitamin E [27] and tocotrienols [28] yielded encouraging results in relation to lifespan extension. Although vitamin E treatment was shown to extend lifespan, it was not clear whether this was a specific effect or a result of hormesis (the beneficial effects arising from exposure to mild stress [29]), as treated populations also showed slowed development and a reduction in fertility. The administration of tocotrienols, while resulting in an increase in resistance to oxidative stress, showed only an increase in mean lifespan but no change in maximum lifespan. Other potential antioxidants have been tested in C. elegans, notably the Ginkgo biloba extract EGb 761 [30]. This extract caused a modest extension in lifespan (8%), as well as an increased resistance to oxidative and thermal stresses; however, a purified component, the flavonoid tamarixin, extended lifespan by 25%.

The most compelling evidence for lifespan extension through enhancement of antioxidant defences comes from the administration of small, synthetic SCMs (SOD/catalase mimetics) in the nematode. These compounds had been shown previously to be effective in a number of disease models in other organisms, including endotoxin-induced acute lung injury, autoimmune encephalomyelitis, stroke and spongiform encephalopathy [13,31–34]. Treatment of nematodes with these compounds elicited considerable increases in both mean and maximum lifespan (Figure 1), with no apparent effects on development and fertility [13]. Importantly, these mimetics (Euk-134 and Euk-8) also rescued the decreased mev-1 mutant lifespan, indicating that they were indeed protecting against damage from ROS. Interestingly, these SCMs appear to be effective only under specific experimental conditions, as others have been unable to demonstrate lifespan extension in the nematode [35] and the housefly [36].

While the Euk compounds have been shown to act as antioxidants in vitro, this has not been conclusively demonstrated in the nematode. We have recently shown that Euk-134- and Euk-8-treated populations display considerable resistance to oxidative stress induced by paraquat [36a]. Treatment throughout development increases survival in response to paraquat from 11% in controls to 90% in Euk-134-treated and 67% in Euk-8-treated populations. Furthermore, it has been demonstrated that these compounds are not toxic to the nematode, having no effect on fertility, development or constitutive heat-shock protein gene expression, and that their action is independent of insulin/IGF-I-like signalling.
**Figure 1 | Lifespan analysis of SCM-treated nematode populations**

Wild-type (wt) populations treated or not with Euk-134 and age-1 mutants were assayed for survival in liquid culture at 20°C. The fraction surviving was ascertained by the presence of touch-provoked movement and pharyngeal pumping. Mean lifespan (±S.E.M.) was increased by Euk-134 administration from 26.74±0.88 days in untreated controls to 52.73±2.07 days in populations treated with 0.05 mM Euk-134 (P<0.0001). Maximum lifespan was increased from 43 days in untreated controls to 77 days with Euk-134 treatment. The age-1 mutation results in a mean lifespan of 46.00±2.19 days and a maximum lifespan of 70 days. Typically, a 40% increase in lifespan was observed after treatment with Euk-134.

While further analysis is required to fully elucidate the mechanism of action of these compounds, such as measurement of post-treatment oxidative damage, measurements of the rate of ROS production and their ability to detoxify ROS in vivo, it appears that these compounds do indeed act to decrease oxidative stress in C. elegans.

These studies demonstrate that pharmacological intervention in the aging process, through enhancing natural antioxidant defences, is a realistic proposition. Other pharmacological interventions that extend lifespan may act through enhancing stress resistance or by alternative mechanisms [37], but overall the number of compounds tested in an aging context has been small. To date, the strategy has been to target those compounds that are likely to impact on known aging pathways, principally because of the labour-intensive nature of these studies. It is likely that the identification of novel antioxidants with greater efficacy in combating ROS and extending lifespan will only be accelerated through the development of high-throughput screening technologies. To this end, we have developed a novel, automated, fluorescence-based assay of nematode survival, which should greatly expedite this process [38]. With such a system it is possible for a normal sized academic laboratory to screen many hundreds of compounds for in vivo antioxidant properties.

While considerable progress has been made in understanding the major genetic influences and signalling pathways that determine lifespan, there has been much less progress in understanding the fundamental molecular mechanisms that lead to age-related decline in function. A loss of protein conformational integrity [17,39,40] and an accumulation of oxidative macromolecular damage may, however, play an important part. It is likely that the application of novel screening technologies for identifying antioxidant, ‘anti-aging’ compounds in C. elegans will generate valuable tools for furthering our understanding of the basic biology of aging and in the development of new interventions in age-related disease.

**References**


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