2-SeCD treatment extends lifespan, improves healthspan and enhances resistance to stress in *Caenorhabditis elegans*†

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Aging is primarily caused by reactive oxygen species (ROS). 2-Selenium-bridged β-cyclodextrin (2-SeCD) is an enzyme with glutathione peroxidase (GPX)-like activity, which indicates that it might be an important ROS scavenger *in vivo*. However, its effects on aging are unknown. In this study, we studied the dose-dependent effects of 2-SeCD on the lifespan, healthspan and resistance to stress of *Caenorhabditis elegans*. The mean lifespan of nematodes treated with 0.05, 0.5, and 5 mM 2-SeCD increased significantly in a dose-dependent manner. Healthspan also improved, as revealed by increased motility and GPX activity and decreased lipid peroxidation and ROS level, indicating that DNA damage was curbed. In addition, nematodes treated with 2-SeCD were more resistant to heat stress, UV irradiation and H$_2$O$_2$-induced oxidative stress. Our findings indicate that 2-SeCD extends the lifespan of *C. elegans*, improves the healthspan and enhances the resistance to stress.

1 Introduction

Age is a major risk factor for many chronic diseases. In the mid-1950s, Denham Harman articulated the free radical theory of aging, speculating that endogenous oxygen radicals, which are generated in cells, cause cumulative damage. One of the major causes of aging is the generation of reactive oxygen species (ROS); thus, any factor that can reduce the generation of ROS may delay senescence. During aerobic respiration, organisms produce ROS when electrons are transferred along the respiratory chain to generate ATP. In a normal system, oxygen is the final electron acceptor, thereby yielding water. However, an inappropriate electron donation, usually involving ubiquinone within complex III, generates ROS. Specifically, ROS are chemical species generated endogenously and exogenously from several different sources, including superoxide anions, hydroxyl radicals and hydrogen peroxide. Several species, such as superoxide anions or hydroxyl radicals, are extremely unstable, whereas others, such as hydrogen peroxide, are freely diffusible and relatively long-lived. Moreover, ROS cause lipid peroxidation, protein oxidation and DNA damage, which are deleterious to cells. A rise in intracellular ROS levels can damage various cell components and trigger specific signaling pathways. Both of these effects can influence numerous cellular processes linked to aging and the development of age-related diseases. It has been shown that treatments that increase oxidative stress promote cellular damage and reduce the lifespan of various species. ROS-induced damage can shorten the lifespan of organisms by a mechanism that is different from normal aging. Reducing intracellular ROS may, in principle, be achieved by increasing antioxidant defenses in the cell, either by dietary supplements of antioxidants or by overexpression of genes encoding antioxidant enzymes.

Selenium is a necessary trace element of the human body, and it plays an important role in human metabolism. A lack or excess of selenium can cause many kinds of diseases. Glutathione peroxidase (GPX) in all eukaryotic cells is the first enzyme of the selenium family. It removes hydrogen peroxide and lipid peroxide, which boosts the metabolism of hydrogen peroxide and protects living organisms from oxidative damage. Many diseases, such as Alzheimer’s disease, immunological diseases, irradiation damage, and their pathogenesis are associated with changes in antioxidant enzyme levels. Most GPX studies focus on its catalytic mechanism and active site. Studies on the artificial simulation can not only help to develop viable drugs, but also help to improve the understanding of GPX structure and catalytic function. Cyclodextrin selenium generated (2-SeCD), known as a GPX simulator, is a synthetic organic compound selenide. It has unique GPX activity, antioxidant and anti-inflammatory properties, with very low toxicity. It also has a hydrophobic cavity, which is similar to the base enzyme...
activity site, which can wrap the substrate, and the end of the hydroxyl can produce a catalytic site. According to a previous report, 2-SeCD is a very promising compound. It can replace ebselen, known as the GPX simulation. Furthermore, it has enhanced enzyme activity and is water-soluble,\(^1\) so it can bind a variety of organic molecules and remove ROS. Recently, GPX has been confirmed to show excessive expression in cancer cells and to antitumor effect.\(^1\) These results suggest that 2-SeCD may be the target of cancer treatment.

Organisms are vulnerable to environmental challenges. With time, the body will deteriorate, increasing the risks of disease and death. Nematodes are good models of aging that are similar with higher mammals, including humans. As they age, they become less fertile, move more slowly and develop sarcoma. They also accumulate oxidants and liposomes, which are common features of aging in many types of.\(^1\) At the nematode and human levels, genes and proteins involved in aging are 80% similar.\(^1\) In addition to biological aging models, nematodes have unique advantages. They are easily cultured and have a short life cycle, allowing rapid replication of experimental treatments. In addition, this model has been successfully applied to pharmacological studies of drugs and interactions between genes. More importantly, vitamins, clinical drugs, antioxidant supplements, and phytochemicals can extend human life and physiological senescence.

Specifically, our objectives were to determine the effects of 2-SeCD on the lifespan, healthspan and stress resistance in vivo using *C. elegans* as a model.

## 2 Materials and methods

### 2.1 Chemicals and reagents

Sodium azide, thiobarbituric acid, glutathione reductase and NADPH were purchased from TransGen Biotech Co., Ltd. (Beijing, China). All other chemicals were of analytical or reagent grade.

### 2.2 Preparation and storage of the 2-SeCD stock solution

Professor Guimin Luo from the Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, Jilin University, Changchun, P. R. China kindly provided the 2-SeCD. The 2-SeCD was stored as a solid at room temperature. A 10 mM stock solution was prepared in distilled water, sterilized through a 0.2 \(\mu\)m filter and stored at 4 °C.

### 2.3 Strain maintenance and nematode culture

*C. elegans* Bristol N2 (wild-type) and PD4251 (\(pSAK2\) myo-3p::GFP::LacZ::NLS + \(pSAK4\) myo-3p::mitochondrial GFP + dpy-20(+)) 1 strains, both of which were available in our laboratory, were used. The PD4251 strain expressed green fluorescent protein (GFP) in all body wall and vulval muscles, whereas at the cellular level, GFP was expressed in nuclei and mitochondria. Both strains were cultured in plates containing nematode growth medium (NGM; 1.7% agar and 2.5 \(\mu\)g ml\(^{-1}\) peptone dissolved in 25 mM KH\(_2\)PO\(_4\), 50 mM NaCl, 5 \(\mu\)g ml\(^{-1}\) cholesterol, 1 mM MgSO\(_4\), and 1 mM CaCl\(_2\)) and the *Escherichia coli* OP50 strain, which served as a food source, as previously described.\(^1\) *Escherichia coli* OP50 is uracil defect and has low metabolic rate, so even if it ate 2-SeCD to produce a metabolite, it was negligible comparing with the amount of 2-SeCD applied to the culture medium. Thus, its effect can be ignored for its effect on nematode worms. The 2-SeCD stock solution was diluted by logarithmic phase *Escherichia coli* OP50 cultured in LB liquid medium to a series concentration. The control group worms were fed with *Escherichia coli* OP50 only.

### 2.4 Lifespan assay

Twenty gravid adult nematodes were cultured on NGM–OP50 plates (control) or NGM–OP50 plates containing 0.05, 0.5, or 5 mM 2-SeCD and allowed to lay eggs at 20 °C for approximately 2 h to obtain a synchronous population. After 2 h, the nematodes were removed, and the plates were placed back at 20 °C until the progeny reached adulthood (~72 h). The nematodes were transferred every other day to new NGM–OP50 plates or NGM–OP50 plates containing 2-SeCD. Nematode viability was scored daily or every other day by gentle prodding with a brow. Animals that failed to move were assumed to be dead and not scored, whereas those that exhibited bagging or crawling, or those that exploded upon prodding were scored (\(n \geq 40\) per group).

### 2.5 Stress resistance assay

Nematodes were grown on NGM–OP50 plates until a late larval stage (i.e., L4, ~96 h after hatching) and subsequently transferred to new NGM–OP50 plates or NGM–OP50 plates containing 0.05, 0.5, or 5 mM 2-SeCD for 48 h, with \(n \geq 40\) animals per treatment group. Thereafter, nematodes were subjected to heat shock, UV irradiation or H\(_2\)O\(_2\) oxidation.

#### 2.5.1 Heat shock

The heat shock experiment was carried out according to the method of Lithgow et al.\(^1\) On the third day of adulthood, nematodes were incubated at 35 °C and monitored every 1 h thereafter until all of the animals had died. The control group consisted of nematodes incubated at 20 °C.

#### 2.5.2 UV irradiation

On the third day of adulthood, nematodes were transferred to bacteria-free NGM plates and subjected to UV irradiation (1000 J m\(^{-2}\) generated by a single 15 watt UV light bulb) for 8 min 40 s. Thereafter, nematodes were transferred to NGM–OP50 plates and monitored daily for survival by gentle prodding with a brow.\(^1\) The control group consisted of 2-SeCD-untreated nematodes subjected to UV irradiation.

#### 2.5.3 H\(_2\)O\(_2\) oxidative stress

The H\(_2\)O\(_2\) oxidative stress experiment was carried out as previously described for the stress resistance assay. On the third day of adulthood, nematodes were transferred to NGM–OP50 plates containing 15 mM H\(_2\)O\(_2\) and monitored daily for survival.\(^1\)

### 2.6 Motility

Nematode motility can be assessed by two approaches; in the first approach, spontaneous movement is assessed, whereas in the second approach, swimming frequency in liquid culture medium is measured. To assess motility in *C. elegans*, we used...
the first approach as previously described.\textsuperscript{19} Nematodes with spontaneous and smooth movement, which formed a sinusoidal and symmetric pattern, were regarded as type A, whereas those with limited movement (i.e., only the nose or tail moved upon gentle prodding with a bow) were regarded as type C. Type B was defined as movement falling in between types A and C.\textsuperscript{20} ($n \geq 40$ per group).

2.7 Assessment of the body wall muscle integrity

To assess body wall muscle integrity, the PD4251 strain was used. Newly hatched nematodes were cultured on NGM-OP50 plates containing 5 mM 2-SeCD. The control group consisted of 2-SeCD-untreated nematodes cultured on NGM–OP50 plates. On the fifth day, nematodes were treated with 1% NaN\textsubscript{3} and transferred onto 5% agarose-coated glass slides. Images were acquired using an inverted fluorescent microscope and intensity was quantified by Image-Pro Plus ($n \geq 8$ per group).

2.8 GPX activity

Nematodes were grown on NGM–OP50 plates from hatching to adulthood, and then incubated with 0.05, 0.5, or 5 mM 2-SeCD for 48 h. After 12 h, the nematodes were harvested and washed thrice in M\textsubscript{s} buffer (22 mM KH\textsubscript{2}PO\textsubscript{4}, 42 mM Na\textsubscript{2}HPO\textsubscript{4}, 85 mM NaCl, and 1 mM MgSO\textsubscript{4}, pH 7.0) to remove residual 2-SeCD.\textsuperscript{21} Glutathione peroxidase activity was determined by the method of Wilson et al.\textsuperscript{22} The nematodes were homogenized in M\textsubscript{s} buffer, and lysates were centrifuged at 8000 g for 10 min at 4 °C. The 500 μl reaction contained 50 μg of lysate, 50 mM NaH\textsubscript{2}PO\textsubscript{4}, 1 mM EDTA, 1 mM NaN\textsubscript{3}, 1 mM glutathione reductase, 0.25 mM NADPH and 1 U glutathione reductase (pH 7.0), and it was carried out at 37 °C. The reaction was initiated by the addition of 0.5 mM H\textsubscript{2}O\textsubscript{2}. The absorbance of NADPH at 340 nm corresponded to glutathione peroxidase activity ($n \geq 40$ per group).

2.9 Lipid peroxidation assay

Nematodes were divided into three groups, with $n \geq 60$ animals per treatment group as follows: (I-control) newly hatched nematodes were cultured on NGM-OP50 plates only, (II-H\textsubscript{2}O\textsubscript{2}) newly hatched nematodes were cultured on NGM-OP50 plates for 144 h, treated with 15 mM H\textsubscript{2}O\textsubscript{2} for 3 h in the dark, and then transferred to NGM–OP50 plates, and (III-H\textsubscript{2}O\textsubscript{2} + 2-SeCD) newly hatched nematodes were cultured on NGM-OP50 plates for 96 h, treated with 5 mM 2-SeCD for 48 h, treated with 15 mM H\textsubscript{2}O\textsubscript{2} for 3 h in the dark, and then transferred to NGM-OP50 plates. Nematodes were harvested at 171 h, suspended in 200 μl of a mixture of 0.8% thiobarbituric acid, 16% trichloroacetic acid and 8.33% concentrated sulfuric acid, and then incubated in a water bath set at 95 °C for 30 min. The absorbance at 532 nm corresponded to the MDA level\textsuperscript{23} ($n \geq 40$ per group).

2.10 Measurement of reactive oxygen species (ROS)

Intracellular reactive oxygen species (ROS) in \textit{C. elegans} were determined by dichlorofluorescin diacetate (DCF-DA). Nematodes were collected at 171 h and treated as described in lipid peroxidation assay. Thereafter, animals were analyzed immediately by a commercially available kit (Beyotime Institute of Biotechnology, China).\textsuperscript{24–26} Samples were read every 20 min for 2 h at excitation 488 nm and emission 525 nm.

2.11 Determination of the brood size

After birth, the nematodes were individually grown on NGM-OP50 plates in the absence (control) or presence of 5 mM 2-SeCD, with 10 animals per treatment group. Thereafter, animals were transferred every 12 h to new NGM-OP50 plates until egg production had ceased. The total number of progeny for each treatment group was calculated\textsuperscript{27} ($n \geq 8$ per group).

2.12 Statistical analysis

Statistical analysis was performed using Prism 4 software (GraphPad Software, Inc., La Jolla, CA, USA). Results were expressed as the mean ± standard deviation of three or four independent experiments. The lifespan of nematodes cultured in the absence or presence of 2-SeCD was compared between groups using the two-tailed, unpaired Student’s t-test. $P$-values < 0.05 were taken as statistically significant ($0.01 \leq *P < 0.05$, 0.001 ≤ **$P < 0.01$, ***$P < 0.001$).

3 Results

3.1 2-SeCD increases the lifespan of \textit{C. elegans}

The reported mean lifespan of wild-type \textit{C. elegans} nematodes is 2–3 weeks at 20 °C, which is consistent with our results (16.58 ± 2.11 days, with a maximum of 21 days). After the treatment of newly hatched nematodes with 0.05, 0.5, or 5 mM 2-SeCD, the mean lifespan was 17.06 ± 2.18 days (with a maximum of 21 days), 17.78 ± 2.70 days (with a maximum of 22 days), and 18.26

Fig. 1. Effect of different concentrations of 2-SeCD on wild type \textit{C. elegans} lifespan from born at 20 °C. Animals showed longevity consist with control in a dose-dependent manner. ($N \geq 40$ per group, 0.01 ≤ *$P < 0.05$, 0.001 ≤ **$P < 0.01$, ***$P < 0.001$).

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2.54 days (with a maximum of 22 days), respectively, indicating that 2-SeCD could significantly affect the lifespan of *C. elegans* (Fig. 1a and b).

### 3.2 2-SeCD increases the resistance of *C. elegans* to stress

Young adult nematodes were treated with 0.05, 0.5, or 5 mM 2-SeCD for 48 h. Thereafter, their responses to a variety of physical and chemical stressors were assessed.

#### 3.2.1 Heat shock

To induce stress by heat shock, nematodes were incubated in the absence (control) or presence of 2-SeCD for 48 h and then incubated at 35 °C until all of the animals had died. Although 69% of control nematodes died after 5 h of heat shock, 51%, 57%, and 75% of nematodes were viable after treatment with 0.05, 0.5, and 5 mM 2-SeCD, respectively (Fig. 2a and b).

#### 3.2.2 UV irradiation

To induce stress by UV irradiation, nematodes were incubated in the absence (control) or presence of 2-SeCD and then exposed to UV irradiation (1000 J m^-2). Nematodes treated with 2-SeCD survived significantly longer than control nematodes (Fig. 2c and d).

#### 3.2.3 H2O2 oxidation

To induce stress by H2O2 oxidation, nematodes were incubated in the absence (control) or presence of 2-SeCD for 48 h, and then cultured on NGM–OP50 plates containing 15 mM H2O2. Although control nematodes survived for an average of 8.47 h, those treated with 0.05, 0.5, or 5 mM 2-SeCD survived for an average of 10.11 h (with a maximum of 18 h), 10.97 h (with a maximum of 18 h), and 12.39 h (with a maximum of 20 h), respectively, representing increases of 19%, 29%, and 46% over control nematodes (Fig. 2e and f).

### 3.3 2-SeCD improves nematode motility

We investigated the motility of 10-, 12- and 14 day-old nematodes treated with 2-SeCD. There was a significant dose-dependent decline in the motility of 10-, 12- and 14 day-old nematodes. Whether animals treated with 2-SeCD or not, most 10 day-old nematodes moved spontaneously, and there was a marked difference in spontaneous motility (type A) between the control and treatment groups. Furthermore, 12 day-old nematodes treated with 0.05, 0.5 and 5 mM 2-SeCD showed increased spontaneous motility (33%, 46% and 62%, respectively) compared with that of the control group (8%). The most
apparent changes occurred in 14 day-old nematodes; 42% of nematodes in the control group barely moved their heads after gently prodding with a brow (type C) compared to the treatment groups (less than 20%), whereas 25% of the nematodes treated with 5 mM 2-SeCD showed spontaneous movement (Fig. 3a–c). To determine if 2-SeCD improves muscle function, we treated the PD4251 strain with 5 mM 2-SeCD and found an increased number of GFP light spots and bright points on the body wall compared to the control, suggesting that 2-SeCD enhances motility by strengthening body wall muscles (Fig. 3d–j).

3.4 2-SeCD increases glutathione peroxidase activity in nematode

We investigated whether 2-SeCD can increase glutathione peroxidase activity and resistance to ROS generators. We found that 2-SeCD increased the glutathione peroxidase activity level. 2-SeCD at 0.05 mM increased total glutathione peroxidase activity levels in whole homogenates from treated worms in the manner of dose-dependence (Fig. 4).

3.5 2-SeCD decreases the lipid peroxidation and ROS level in nematode

The lipid peroxidation product, MDA, is often used to measure lipid peroxidation. We found that the MDA level was higher in H$_2$O$_2$-treated nematodes than in control nematodes. Due to the
To determine whether 2-SeCD affects fecundity, we measured the brood size of nematodes cultured in the absence (control) or presence of 0.05, 0.001, <p>0.05, 0.001 <p>0.01, ***p < 0.001).

3.6 2-SeCD does not affect the nematode brood size

To determine whether 2-SeCD affects fecundity, we measured the brood size of nematodes cultured in the absence (control) or presence of 0.05, 0.001, or 5 mM 2-SeCD. There was no significant difference in the brood size between the control and treatment groups (Fig. 6).

4 Discussion

Several studies have shown that 2-SeCD has glutathione peroxidase-like activity. In this study, we examined the effects of 2-SeCD on several key parameters in *C. elegans*. We found that 2-SeCD was non-toxic at the concentrations used in this study.

Our key findings were that dietary 2-SeCD enhanced the lifespan of nematodes, increased their resistance to environmental stress in a dose-dependent manner, increased their motility, as well as increased GPX activity and decreased lipid peroxidation and ROS level.

We first investigated the effect of 2-SeCD on worms and found it could extend lifespan and have no toxicity to animals in suitable dose. Then we measured the brood size of animals treated with 2-SeCD and found that there was no difference between the groups, indicating that 2-SeCD was non-toxic to *C. elegans*. Next, we investigated whether 2-SeCD had similar effects in nematodes as those in other species. For the lifespan and brood size assays, worms were treated with 2-SeCD since eggs, and for the stress resistance assay since L4. It was because that lifespan and brood size assays were to reflect the effects of 2-SeCD on development and reproduction, however, it was necessary to ensure animals health statement before stresses. The results were all positive. We found that 2-SeCD not only improved resistance to heat shock, UV irradiation and oxidation induced by H₂O₂, but also extended the lifespan and benefited the healthspan of nematodes, especially their motility. We also used the PD4251 strain to investigate muscle integrity and age-dependent muscle degeneration or sarcopenia. The transgenic *C. elegans* strain (PD4251) expressing green fluorescent protein (GFP)-MYO-3, localized in body wall muscles and vulval muscle nuclei, was fed with 2-SeCD, and muscle integrity was analyzed by quantification of GFP fluorescence. Animals treated with 2-SeCD expressed more GFP than the control.

Moreover, to investigate the effects of 2-SeCD on oxidation in nematodes, we measured GPX activity, lipid peroxidation level and ROS level. 2-SeCD improved GPX activity and reduced the MDA level, which was consistent with our earlier results and may explain why 2-SeCD improved the lifespan and healthspan of *C. elegans*. Oxidations in lipid peroxidation level and ROS level assay were all induced by 15 mM H₂O₂. Glutathione peroxidase is one of the most important enzymes in aerobic organisms, and it can scavenge free radicals and hydrogen peroxide. Selenium, in the form of selenocysteine, is a necessary component of GPX activity. With aging, nematodes became slow moving and finally inactive. After the treatment with various stressors, nematodes quickly aged or died. However, nematodes treated with 2-SeCD exhibited a longer lifespan, more rigorous movement and stronger resistance to stress than untreated nematodes. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. Under a normal physiological state, the body content of MDA is very low, and its content can reflect the level of body cells which are attacked by free radicals. It has been reported that 2-SeCD has widespread effects in other models. For example, Mu and coworkers showed that 2-SeCD protects keratinocytes from apoptosis, inhibits lipid peroxidation, and scavenges ROS. Likewise, Sun and colleagues showed that 2-SeCD decreased UVB irradiation-induced p53 and Bcl-2 levels. Another research group demonstrated that 2-
SeCD might be useful during liver surgery.\textsuperscript{34} \textit{C. elegans} represents a useful model in aging research because nematodes decline behaviorally and physiologically with age in a manner similar to that of higher mammals, including humans.\textsuperscript{35} It is also a widespread model used to study human pharmacological interventions, ranging from vitamins and clinical drugs to antioxidant supplements and phytochemicals.\textsuperscript{36} The results showed that 2-SeCD enhanced GPX activity and reduced lipid peroxidation and ROS levels.

It is known that specific human pharmacological interventions, including vitamins, clinical drugs, antioxidant supplements and phytochemicals, can extend the lifespan or delay physiological aging in \textit{C. elegans}.\textsuperscript{37} For example, extracts of \textit{Ginkgo biloba},\textsuperscript{38} and components of blueberry polyphenols,\textsuperscript{39} extend the lifespan and healthspan of nematodes, and enhance stress resistance through a variety of mechanisms. Despite these and other studies, however, surprisingly little is known about the possible anti-aging effects of 2-SeCD \textit{in vivo}, and this was precisely our study goal. As for the mechanism of action of 2-SeCD, we speculate that it is involved in the signaling pathways associated with aging. Further studies are needed to address the mechanism of action. Moreover, we intend to explore the effects of 2-SeCD in higher animals, which should strengthen the foundation of this GPX-like compound in research and development.

5 Conclusions

In summary, we demonstrated that 2-SeCD, which was nontoxic at the concentrations used in this study, extended the lifespan of \textit{C. elegans}. Nematodes treated with 2-SeCD were more resistant to heat, UV irradiation, and H\textsubscript{2}O\textsubscript{2}-induced oxidative stress, indicating that cellular defenses and immunological parameters were improved. In addition, results from experiments that measured motility, GPX activity and the MDA level revealed that 2-SeCD improved the healthspan of \textit{C. elegans}. Further studies are needed to elucidate the mechanisms behind these health benefits.

Conflicts of interest

There are no conflicts to declare.

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References

29 Q. Yan, R. Zhao, W. Zheng, C. Yin, B. Zhang and W. Ma, BMC Biotechnol., 2009, 9, 47.