*Anti-Aging Medical Research. Vol. 2, No. 1:39- 47, Autumn 2005*

# A superoxide dismutase/catalase mimetic extends the lifespan of short-lived *mev-1* mutant but not the wild type strain in *Caenorhabditis elegans.*

Satoshi Uchiyama, Hideo Koike, Takahiko Shimizu, Takuji Shirasawa

*Department of Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology. Japan*

## Abstract

***Objective:*** Some studies reported lifespan extension of *Caenorhabditis elegans* by treatment with antioxidants, including a SOD/catalase mimetic, EUK-8. Melov et al. reported that treatment with EUK-8 extended the lifespan of wild type N2. On the other hand, treatment with EUK-8 shortened the lifespan of N2 in Keaney's report. In the present study, we investigated the effect of EUK-8 on the lifespan and the resistance to paraquat-induced oxidative stress in C. *elegans.*

***Methods:*** We cultured N2 and three mutants, *clk-1, daf-2* and *mev-1* in liquid culture medium with or without EUK-8, ranging from 0.05 to 0.5 mM. On every second day, we counted the living worms and transferred the worms into fresh medium. In the paraquat study, we measured the survival time of EUK-8-pretreated worms in liquid culture medium with 0.4 M paraquat.

***Results:*** Treatment with 0.5 and 5 mM EUK-8 significantly shortened the lifespan of N2 in a dose-dependent manner. On the other hand, treatment with EUK-8 significantly enhanced the resistance to paraquat-induced oxidative stress in the N2 strain. Treatment with EUK-8 extended the lifespan of a short-lived *mev-1* mutant at a lower dose and shortened the lifespan at a higher dose, although no effect was observed in the long-lived *daf-2* and *clk-1* mutants. These results indicated that EUK-8 effectively protected against reactive oxygen species (ROS) injuries under pathological conditions such as paraquat treatment and *mev-1* mutation, but not under the physiological conditions.

***Conclusion:*** Our results suggested that the balance between the production and degradation of ROS may control the lifespan of *C. elegans.*

***Key words:*** reactive oxygen species, superoxide dismutase/catalase mimetic, EUK-8, *Caenorhabditis elegans,* oxidative stress, lifespan

Received: 2005. 5. 7
Accepted: 2005. 8.31
Reprint requests to: Takuji Shirasawa, M.D, Department of Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan. Tel: +813-3964-3241 Fax: +813-3579-4776
e-mail: sirasawa@tmig.or.jp

## Introduction

It is well known that the accumulation of molecular damage by free radicals, especially reactive oxygen species (ROS), is a major cause of aging.[1] ROS can modify endogenous molecules such as lipids, proteins and DNAs. Most organisms have developed antioxidant defense systems that include antioxidant enzymes and small antioxidants. Superoxide dismutase (SOD) catalyses the dismutation of $O_2^-$ to $H_2O_2$, and then $H_2O_2$ is further reduced to $H_2O$ by catalase or glutathione peroxidase. A large number of studies have focused on the relationship between aging and oxidative stress1. Many studies have shown an age-related accumulation of oxidative damage in lipids, proteins and DNA in invertebrates and vertebrates.[1,2]

Genomically defined non-mammalian model organisms, such as nematode, fruitfly and yeast are the powerful experimental systems for biological research. These model organisms have also been studied for the molecular mechanisms of aging and age-related diseases.[3] The free-living nematode, *Caenorhabditis elegans*, is widely used for aging research, because the mean lifespan of *C. elegans* is reported to be approximately 20 days at 20° C.[4] Furthermore, a variety of mutagenesis methods provide potential advantages for genetic studies in *C. elegans*.[4,5] Actually, a variety of mutants with age-related phenotypes have been isolated and the causal genes responsible for their phenotypes have been identified.[6,7] Since the transparency in the body of *C. elegans* allows the observation of visible process as in aging, such as the increase of lipofuscin-like granules[4], we can easily screen compounds, which control the lifespan and aging process in *C. elegans*.

A large number of long-lived mutants have been reported in *C. elegans*.[3] Long-lived mutant worms had an enhanced resistance to several stresses, including heat, UV-irradiation and ROS.[8] Mutation of the *daf-2* gene, which encodes a homolog of the insulin/IGF-1 receptor family, increases the lifespan and upregulates the expression of antioxidant enzymes such as MnSOD.[9] Mutation of the *clk-1* gene, which encodes a hydroxylase involved in the biosynthesis of coenzyme Q (CoQ), results in decreased endogenous CoQ9, and increased lifespan.[10] Several groups have successfully used the increased stress resistance to heat or oxidative stress to identify a long-lived mutant in *C. elegans*.[11] On the other hand, the mutation of the *mev-1* gene, which encodes a subunit of the enzyme succinate dehydrogenase cytochrome b, results in the overproduction of superoxide and a shortened lifespan.[12] These reports indicate the correlation between lifespan and resistance to oxidative stress.

Some reports demonstrated the extension of lifespan by enhancing antioxidative defense by the exogenous administration of antioxidant in *C. elegans*. $CoQ_{10}$ and vitamin E extended the lifespan of the wild type worm.[13] EGb761, the extract of the *Ginkgo biloba* leaves, and the flavonoid tamarixetin, the component of EGb761, increased median lifespan by 8% and 25%, respectively.[14] These results raise the possibility that exogenous administration of antioxidants can prolong the lifespan by lowering oxidative stress.

Interestingly, the discrepancy in the effect of EUK-8, a SOD/catalase mimetic, on the *C. elegans* lifespan has been reported in previous reports.[15,16,17] In 2000, Melov and coworkers reported the extension of *C. elegans* lifespan by the administration of SOD/catalase mimetics, EUK-8 or EUK-134, demonstrating that ROS is a major factor in limiting lifespan.[15] On the other hand, Keaney et al. reported the shortening of lifespan due to the administration of EUK-8 in a dose-dependent manner, in spite of using the same concentration range as in Melov's report.[16,17]

In the present study, we investigated the effect of EUK-8 on lifespan using *C. elegans* strains, wild type N2 and three mutant worms, a short-lived *mev-1* mutant, a long-lived *daf-2* mutant and a long-lived *clk-1* mutant. Here, we demonstrate that the lifespans of the N2 and mutant worms were affected by treatment with EUK-8 in different manner.

## Materials and Methods

*C. elegans strains*

We employed wild type N2, *clk-1 (e2519), daf-2 (e1370)* and *mev-1 (kn1)* in this study. N2, *clk-1 (e2519)* and *daf-2 (e1370)* were provided by the Caenorhabditis Genetic Center (University of Minnesota, St. Paul, MN). *mev-1* mutant was provided by Dr. N. Ishii (Tokai University, Kanagawa, Japan).

## Measurement of lifespan of worms

We measured the lifespan of *C. elegans* with or without EUK-8 (Calbiochem, San Diego, CA, USA) according to the report by Keaney et al.[16] We prepared experimental worms with synchronized culture. Briefly, we collected eggs by alkaline hypochlorite extraction of gravid hermaphrodites. Eggs were allowed to hatch by incubating at 20° C in S-basal. L1 larvae were incubated at 20° C in S

medium with *Escherichia coli* OP-50 as a food source. We added EUK-8 into the culture medium after the worms had reached the L4 stage. We transferred the worms into fresh culture medium every second day and counted the living worms at the time of transfer. We analyzed the mortality data with Kaplan-Meier survival analysis. The lifespans of the EUK-8 treated worms were compared to the untreated worms using the log rank test.

## Paraquat treatment

We prepared experimental worms as described above. We synchronously cultured worms at the age of 5 days in culture medium with 0.05 and 0.5 mM EUK-8 at 20° C. After treatment with EUK-8 for 2 days, we washed the worms in distilled water three times and incubated the worms in S medium with 0.4 M paraquat, 1,1'-dimethyl-4, 4'-bipyridinium dichloride (SIGMA, St Louis, MO, USA), at 20° C. We counted the living worms every hour. We analyzed the mortality data using Kaplan-Meier survival analysis. The lifespans of the EUK-8 treated worms were compared to the untreated worms using the log rank test.

## Results

### Relationship between lifespan and paraquat resistance of *C. elegans*

*C. elegans* is a useful model for aging research. N2, a wild type worm lives for an average of 20 days under laboratory conditions. A large number of lifespan-related mutants have been identified and these mutant worms demonstrate extended or shortened lifespans.[1,8] In this study, we used wild type N2 and three mutants, short-lived *mev-1*, long-lived *clk-1*, and long-lived *daf-2* mutants to investigate the effect of EUK-8 on the lifespan of *C. elegans*, because these mutants show different sensitivities to oxidative stress.[9,12] It has been reported that mutation of the *mev-1* gene resulted in an overproduction of the superoxide anion and a shortened lifespan.[12] On the other hand, mutation of the *daf-2* gene reveals the longevity and resistance to oxidative stress.[9] Mutation of the *clk-1* gene results in decreased endogenous CoQ9 and an increased lifespan.[10] To confirm the reproducibility of their phenotype in our experimental condition, we measured the lifespan and resistance to paraquat, a superoxide generator, in these worms. As shown in Fig. 1, *daf-2* and *clk-1* mutants showed an extended lifespan and resistance to paraquat-induced oxidative stress (Fig. 1A and 1B). Furthermore, *mev-1* showed a shortened lifespan and increased sensitivity to paraquat-induced oxidative stress (Fig. 1). Our results are consisted with previous reports of *mev-1, daf-2* and *clk-1* mutants. These data indicate a positive correlation between the lifespan and resistance to oxidative stress.

### Effect of EUK-8 on the lifespan and paraquat resistance of *C. elegans*

It is well known that oxidative stress is a major cause of aging. Long-lived mutant worms are resistant to a variety of stresses, including oxidative stress (Fig.1). Previous studies on *C. elegans* demonstrated an extension of lifespan due to the administration of antioxidants such as EGb761, and $CoQ_{10}$.[13,14] In 2000, Melov and coworkers reported the lifespan extension of *C. elegans* due to the administration of the SOD/catalase mimetics, EUK-8 and EUK-134.[15] Melov's report indicated the possibility that antioxidants can prolong lifespan by lowering the ROS level; however, another group reported the toxic effect of EUK-8 with no increase of lifespan of *C. elegans*.[16,17]

To confirm the effect of the EUK-8 on lifespan of *C. elegans*, we measured the lifespan of *C. elegans* in liquid culture medium with or without EUK-8. Administration of EUK-8 showed the different effects on lifespan of *daf-2, clk-1, mev-1* and N2. No significant effect was observed on the lifespan of N2 treated with 0.05 mM EUK-8 ($p > 0.05$, Fig. 2A, 3A and Table 1), and treatment with EUK-8 at higher doses of 0.5 and 5 mM significantly shortened the mean lifespan of N2 in a dose dependent manner (9% and 64% decrease, $p < 0.05$ and $< 0.001$, respectively, Fig. 2A, 3A and Table 1). On the other hand, EUK-8 administration to N2 resulted in an increase of paraquat resistance in a dose dependent manner (Fig. 3B and Table1). We obtained very similar results in an independent experiment (data not shown). These results indicate that EUK-8 has a toxic effect on the lifespan and a protective effect against acute oxidative stress in wild type N2.

Long-lived *daf-2* and *clk-1* mutants are more resistant to a variety of stresses, including oxidative stress (Fig.1). Some reports showed the enhanced expression of antioxidant enzyme genes, such as MnSOD,[9] and the evidence for the decreased oxidative damage in *daf-2*.[2] We investigated whether EUK-8 treatment increases the lifespan of *daf-2* and *clk-1* mutants. As shown in Fig. 2A, 3A and Table 1, the lifespan of *daf-2* was decreased due to the treatment with 0.05 and 0.5 mM EUK-8, although no

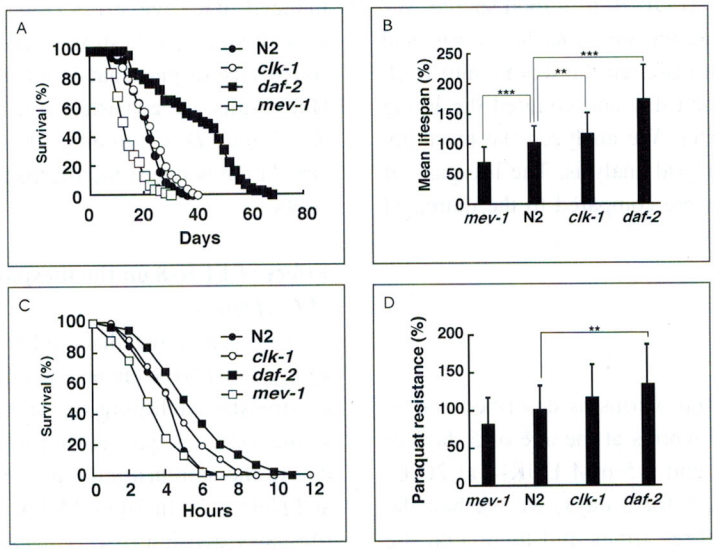


**Figure 1**. Lifespan and resistance to paraquat-induced oxidative stress in *C. elegans* strains. Survival curves (A) and mean lifespan (B) of wild type N2 and mutant strains, *clk-1 (e2519), daf-2 (e1370)* and *mev-1 (kn1)* under normal conditions. Worms were grown in culture medium at 20° C as described in the Methods section. Survival curves (C) and mean survival time (D) of N2, *clk-1 (e2519), daf-2 (e1370)* and *mev-1 (kn1)* treated with 0.4 M paraquat as described in the Methods section. Error bars represent standard deviation. ** $p < 0.01$ and *** $p < 0.001$. *P* value log rank as compared with N2.

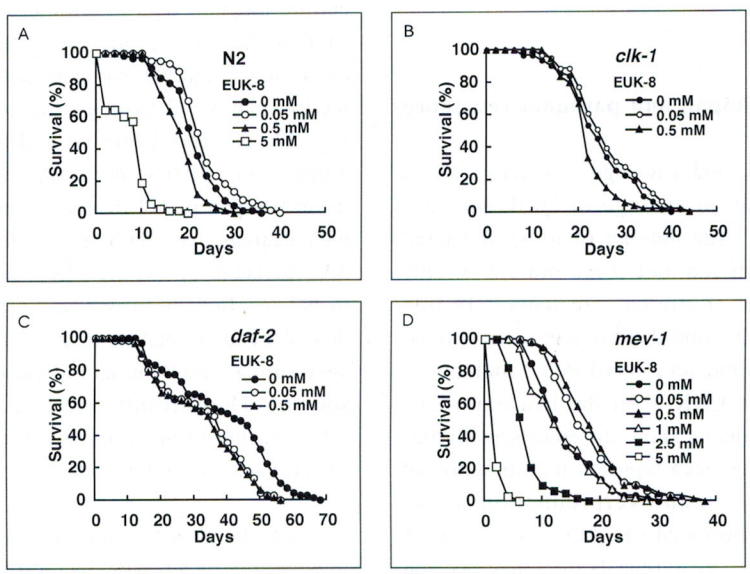


**Figure 2**. Effect of EUK-8 on the lifespan of *C. elegans* strains. (A) Lifespan of N2 treated with 0.05 0.5 and 5 mM EUK-8. (B) Lifespan of *clk-1 (e2519)* treated with 0.05 and 0.5 mM EUK8 (C) Lifespan of *daf-2 (e1370)* treated with 0.05 and 0.5 mM EUK-8. (D) Lifespan of *mev-1 (kn1)* treated with 0.05, 0.5, 1, 2.5 and 5 mM EUK-8. Worms were grown in liquid culture with or without EUK-8 at 20° C as described in the Methods section. These data are summarized in Table 1.

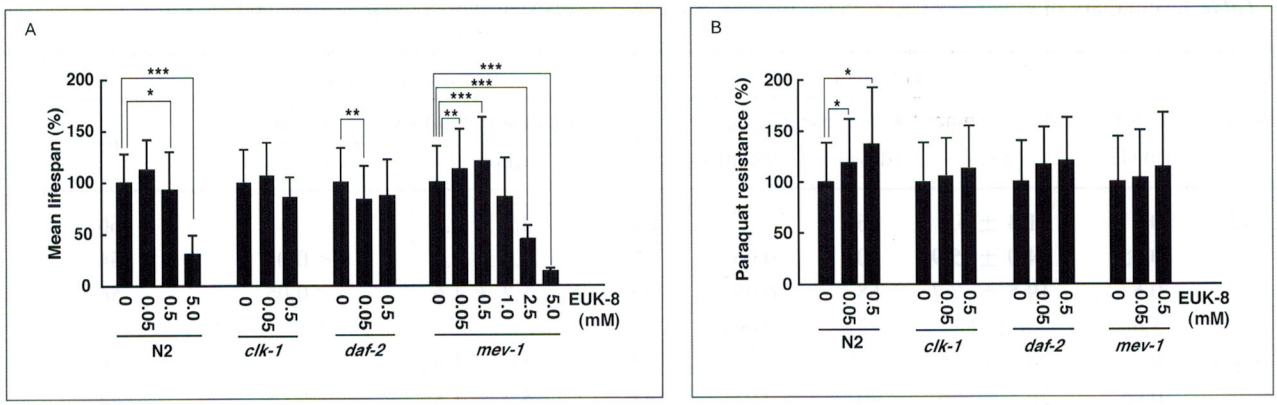


**Figure 3**. Effect of EUK-8 on the lifespan and resistance to paraquat-induced oxidative stress in *C. elegans* strains. (A) Mean lifespan of N2, *clk-1 (e2519), daf-2 (e1370)* and *mev-1 (kn1)* treated with EUK-8. (B) Mean survival time of N2, *clk-1 (e2519), daf-2 (e1370)* and *mev-1 (kn1)* strains incubated in 0.4 M paraquat. These worms were pretreated with the indicated concentration of EUK-8 for 2 days as described in the Methods section. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. *P* value log rank as compared with untreated worms. Error bars represent standard deviation. These data are summarized in Table 1.

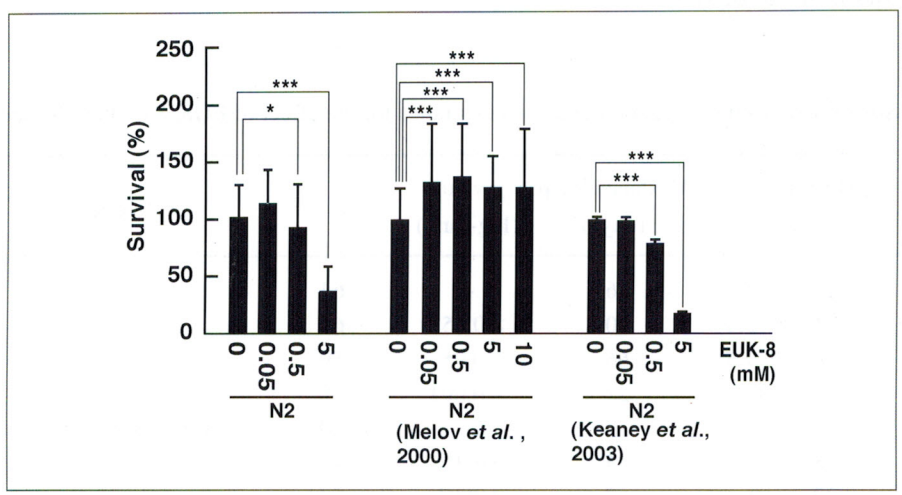


**Figure 4**. Effect of EUK-8 on lifespan of N2 in this study and previous studies. Our data are compared to the data represented in Melov *et al.* and Keaney *et al.*. * $p < 0.05$ and *** $p < 0.001$. *P* value log rank as compared with untreated worms. Error bars in our results and Melov's results represent standard deviation, ann Keaney's report represent standard error. These data are summarized in Table 2.

***Table 1.*** Summary of effect of EUK-8 on the lifespan and paraquat resistance of N2, *daf-2, clk-1* and m*ev-1.*

| Strain | EUK8 (mM) | Lifespan | | | | Paraquat resistance | | |
|---|---|---|---|---|---|---|---|---|
| | | Mean lifespan[a] (days) | Maximum lifespan (days) | *p* value[b] (log-rank) | n | Mean survival time[a] (hours) | *p* value[b] (log-rank) | n |
| N2 | 0 | 21.4 ± 5.7 | 36 | | 64 | 4.3 ± 1.4 | | 60 |
| | 0.05 | 24.0 ± 5.9 | 40 | > 0.05 | 60 | 5.0 ± 1.6 | < 0.05 | 54 |
| | 0.5 | 19.5 ± 7.7 | 68 | < 0.05 | 59 | 5.2 ± 1.6 | < 0.05 | 56 |
| | 5.0 | 7.6 ± 4.7 | 22 | < 0.001 | 68 | | NT[c] | -- |
| *clk-1* | 0 | 24.7 ± 7.5 | 44 | | 62 | 5.0 ± 1.9 | | 46 |
| | 0.05 | 26.3 ± 7.6 | 40 | > 0.05 | 61 | 5.2 ± 1.8 | > 0.05 | 44 |
| | 0.5 | 21.8 ± 5.5 | 34 | > 0.05 | 51 | 5.5 ± 2.0 | > 0.05 | 42 |
| *daf-2* | 0 | 36.9 ± 12.2 | 64 | | 57 | 5.7 ± 2.3 | | 44 |
| | 0.05 | 30.4 ± 12.1 | 66 | < 0.01 | 56 | 6.7 ± 2.1 | > 0.05 | 45 |
| | 0.5 | 31.9 ± 12.7 | 62 | > 0.05 | 55 | 6.9 ± 2.4 | > 0.05 | 38 |
| *mev-1* | 0 | 14.4 ± 5.3 | 30 | | 61 | 3.5 ± 1.5 | | 45 |
| | 0.05 | 18.8 ± 6.0 | 34 | < 0.01 | 61 | 3.7 ± 1.6 | > 0.05 | 45 |
| | 0.5 | 19.1 ± 6.1 | 38 | < 0.001 | 61 | 4.0 ± 1.9 | > 0.05 | 45 |
| | 1.0 | 13.7 ± 5.6 | 28 | > 0.05 | 54 | NT | - | - |
| | 2.5 | 7.6 ± 3.0 | 18 | < 0.001 | 63 | NT | - | - |
| | 5.0 | 2.5 ± 1.0 | 6 | < 0.001 | 70 | NT | - | - |

[a]Data represent the mean ± SD.
[b]*P* value as compared with untreated worms.
[c]NT, not tested.

***Table 2.*** Comparison of data from the previous and present studies for the effect of EUK-8 on the lifespan of N2.

| Strain | EUK8 (mM) | Mean lifespan[a] (days) | Maximum lifespan (days) | *p* value[b] (log-rank) | n | Reference |
|---|---|---|---|---|---|---|
| N2 | 0 | 21.4 ± 5.7 | 36 | - | 64 | |
| | 0.05 | 24.0 ± 5.9 | 40 | > 0.05 | 60 | |
| | 0.5 | 19.5 ± 7.5 | 66 | < 0.05 | 59 | |
| | 5 | 7.6 ± 4.7 | 22 | < 0.001 | 68 | |
| N2 | 0 | 21 ± 6 | 39 | - | 70 | Melov et al. 2000 |
| | 0.05 | 28 ± 11 | 44 | 0.0001 | 44 | |
| | 0.5 | 29 ± 10 | 57 | 0.0001 | 57 | |
| | 5 | 27 ± 6 | 54 | < 0.0001 | 54 | |
| | 10 | 27 ± 11 | 58 | < 0.0001 | 58 | |
| N2 | 0 | 21.3 ± 0.5 | 27 | | 63 | Keaney et al. 2003 |
| | 0.05 | 21.1 ± 0.5 | 30 | 0.8084 | 75 | |
| | 0.5 | 16.8 ± 0.5 | 27 | < 0.0001 | 86 | |
| | 5 | 3.6 ± 01 | 11 | < 0.0001 | 102 | |

[a]Data represent the mean ± SD.
[b]*P* value as compared with untreated worms.

significant difference was observed in resistance to paraquat in *daf-2* by treatment with 0.05 and 0.5 mM EUK-8. On the other hand, no significant difference was observed in the lifespan and resistance to paraquat in the *clk-1* mutant due to treatment with EUK-8.

The short-lived *mev-1* mutant shows shortened lifespan and rapid accumulation of aging markers such as protein carbonyls.[2,12] Melov *et al.* reported the extension of lifespan due to treatment with 0.5 mM EUK-134. We investigated whether exogenous administration of EUK-8 increases the lifespan of *mev-1* by lowering the endogenous ROS. As shown in Fig. 2D, 3A and Table 1, the lifespan of *mev-1* was increased due to treatment with 0.05 and 0.5 mM EUK-8, in agreement with the result of EUK-134 administration to *mev-1* in Melov's report.[15] However, our data showed the shortening of lifespan of *mev-1* at 1 and 5 mM EUK-8 in a dose dependent manner to the same extent as in N2. No significant difference was observed in the effect of EUK-8 on the resistance to paraquat in *mev-1*. These results suggested that the excess of ROS removal by the higher dose of EUK-8 leads to a shortening of lifespan in *C. elegans*.

## Discussion

In this study, we examined the effect of EUK-8, a SOD/catalase mimetic, on the lifespan of *C. elegans* strains. If ROS limits the lifespan under normal conditions, treatment with EUK-8 should increase the lifespan of N2, a wild type worm. We demonstrated that EUK-8 administration shortened the lifespan of N2 in a dose-dependent manner, although EUK-8 treatment increased the resistance to paraquat-induced oxidative stress in a dose-dependent manner (Fig. 2A, 3A and Table 1). These results are similar to the results in Keaney's report.[16,17] Keaney et al. reported a shortened lifespan and reduced fertility of N2 dose-dependently (Fig. 4, Table 2 and Keaney et al.[16]). Our results and Keaney's results indicate that treatment with EUK-8 does not increase the normal lifespan of *C. elegans*. In the housefly, treatment with EUK-8 also decreased the lifespan in dose-dependent manner.[18] These results suggest the possibility that the toxicity of EUK-8 is not species-specific. In an earlier study, Melov *et al.* reported the lifespan extension of N2 without reduced fertility with EUK-8 at the same dose range as that used in our study (Fig. 4, Table 1 and Melov et al.[15]). The reason for the discrepancy between our study and Melov's study remains unclear. The discrepancy in these results may be caused by the difference in their culture conditions.

To further examine the effect of EUK-8 on the lifespan of *C. elegans*, we used a short-lived *mev-1* mutant to investigate whether exogenously added EUK-8 removes endogenous ROS, such as the superoxide anion, *in vivo*. It has been reported that mutation of the *mev-1* gene resulted in an elevated accumulation of oxidative damage, an increased sensitivity to oxygen and a shortened lifespan (Fig.1 and Ishii *et al.*[12]). Our data showed that the administration of 0.05 and 0.5 mM EUK-8 increased the lifespan of *mev-1*, suggesting that EUK-8 can decrease superoxide *in vivo* (Fig. 2D, 3A and Table1). Melov *et al.* also reported that administration of 0.5 mM EUK-134, an analog of EUK-8, restored a normal lifespan to the *mev-1* mutant by increasing its lifespan.[15] Furthermore, the lifespans of sod2 null mice were partially restored by administration of EUK-8 and EUK-134.[19] In *C.elegans*, EUK-8 is concentrated within the mitochondria in the treated worms*17*. Our data and previous results indicate that EUK-8 can act as a SOD/catalase mimetic *in vivo*, especially in mitochondria. No positive effects on the lifespan were observed in the long-lived mutants, *daf-2* and *clk-1*, treated with EUK-8. These results suggest that the regulatory pathway of lifespan in long-lived mutants, *daf-2* and *clk-1*, is partially overlapped with the effect of EUK-8 on lifespan. In other words, EUK-8 cannot increase the lifespan of *daf-2* and *clk-1* because the ROS level in these mutants is already maintained at lower level than in N2 and *mev-1*. Our results indicated that EUK-8 at a lower dose increased the lifespan under particular conditions, such as the overproduction of superoxide and treatment with an oxidative stress inducer.

Many disease processes are associated with the overproduction of ROS. Many studies reported that synthetic low molecular weight SOD mimetics are effective in protecting against ROS-mediated disease.[20] EUK-8 has shown efficacy in several animal models of human disease, such as familial amyotrophic lateral sclerosis and ischemia-reperfusion injury.[20,21] These studies and our results indicate that EUK-8 can effectively remove superoxide under pathophysiological conditions as well as in the *mev-1* mutant worm.

In the present study, treatment with 0.05 and 0.5 mM EUK-8 extended the lifespan of *mev-1*, although EUK-8 administration at a higher dose resulted in a shortened

lifespan of *mev-1* in a dose-dependent manner without a restoration of the lifespan to wild type N2. Similar results were observed in N2 treated with 2 mM paraquat.[17] These results suggest the possibility that the ROS level of N2 is optimal to the normal life of *C. elegans*, whereas the depletion of ROS in treated worms may result in a shortening of the lifespan. ROS can damage proteins, membranes and DNA; however, many studies have reported that ROS functions as a part of intracellular signaling.[22] For example, Huang *et al.* reported that extracellular signal-related protein kinase (ERK) activation induced by peroxisome proliferator-activated receptor-gamma was blocked by a SOD mimetic, MnTBAP, and induced by superoxide generating systems.[23] If ROS plays a role in a signaling pathway or other biological events in *C. elegans*, the depletion of intracellular ROS may lead to the decrease of lifespan due to cellular dysfunction in worms.

We here demonstrate the harmful effects of treatment with EUK-8 on the lifespan of *C. elegans*. Our results suggest the importance of the balance between production and degradation of ROS in vivo. The consequence of imbalance may result in cellular dysfunction caused by superoxide-driven damage and/or superoxide depletion-mediated damage. Our data suggests the importance of the intracellular ROS level.

**Acknowledgments**

We thank Dr. S. Honda, Dr. Y. Honda and Dr. M. Takahashi for their technical advice. N2, *clk-1 (e2519)* and *daf-2 (e1370)* were provided by the Caenorhabditis Genetic Center. *mev-1 (kn-1)* mutant was provided by Dr. N. Ishii.

## References

1. Bokov A, Chaudhuri A, Richardson A. The role of oxidative damage and stress in aging. *Mech Ageing Dev* 2004;125:811-26.
2. Ishii N, Goto S, Hartman PS. Protein oxidation during aging of the nematode *Caenorhabditis elegans. Free Radic Biol Med* 2002;33:1021-5.
3. Warner HR. Longevity genes: from primitive organisms to humans. *Mech Ageing Dev* 2005;126:235-42.
4. Riddle DL, Blumenthal T, Meyer BJ, James R. Priess, ed. *C. elegans* II. New York: Cold Spring Harbor Laboratory Press, 1997.
5. Liu LX, Spoerke JM, Mulligan EL, Chen J, Reardon B, Westlund B et al. High-throughput isolation of *Caenorhabditis elegans* deletion mutants. *Genome Res* ; 1999;9:859-67.
6. Friedman DB, Johnson TE. Three mutants that extend both mean and maximum life span of the nematode, *Caenorhabditis elegans*, define the age-1 gene. *J Gerontol* 1988;43:B2-9.
7. Lakowski B, Hekimi S. Determination of life-span in *Caenorhabditis elegans* by four clock genes. *Science* 1996;272:1010-3.
8. Honda Y, Honda S. Oxidative stress and life span determination in the nematode *Caenorhabditis elegans. Ann N Y Acad Sci* 2002;959:466-74.
9. Honda Y, Honda S. The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans. Faseb J* 1999;13:1385-93.
10. Miyadera H, Amino H, Hiraishi A, Taka H, Murayama K, Miyoshi H et al. Altered quinone biosynthesis in the long-lived *clk-1* mutants of *Caenorhabditis elegans. J Biol Chem* 2001;276:7713-6.
11. de Castro E, Hegi de Castro S, Johnson TE. Isolation of long-lived mutants in *Caenorhabditis elegans* using selection for resistance to juglone. *Free Radic Biol Med* 2004;37:139-45.
12. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N et al. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. *Nature* 1998;394:694-7.
13. Ishii N, Senoo-Matsuda N, Miyake K, Yasuda K, Ishii T, Hartman PS et al. Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. *Mech Ageing* Dev 2004;125:41-6.
14. Wu Z, Smith JV, Paramasivam V, Butko P, Khan I, Cypser JR et al. Ginkgo biloba extract EGb 761 increases stress resistance and extends life span of *Caenorhabditis elegans. Cell Mol Biol (Noisy-le-grand)* 2002;48:725-31.
15. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE et al. Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 2000; 289:1567-9.
16. Keaney M, Gems D. No increase in lifespan in *Caenorhabditis elegans* upon treatment with the superoxide dismutase mimetic EUK-8. *Free Radic Biol Med* 2003;34:277-82.
17. Keaney M, Matthijssens F, Sharpe M, Vanfleteren J, Gems D. Superoxide dismutase mimetics elevate superoxide dismutase activity in vivo but do not retard aging in the nematode *Caenorhabditis elegans. Free Radic Biol Med* 2004;37:239-50.

18. Bayne AC, Sohal RS. Effects of superoxide dismutase/catalase mimetics on life span and oxidative stress resistance in the housefly, *Musca domestica. Free Radic Biol Med* 2002;32:1229-34.
19. Melov S, Doctrow SR, Schneider JA, Haberson J, Patel M, Coskun PE et al. Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase-catalase mimetics. *J Neurosci* 2001;21:8348-53.
20. Muscoli C, Cuzzocrea S, Riley DP, Zweier JL, Thiemermann C, Wang ZQ, et al. On the selectivity of superoxide dismutase mimetics and its importance in pharmacological studies. *Br J Pharmacol* 2003;140: 445-60.
21. Jung C, Rong Y, Doctrow S, Baudry M, Malfroy B, Xu, Z. Synthetic superoxide dismutase/catalase mimetics reduce oxidative stress and prolong survival in a mouse amyotrophic lateral sclerosis model. *Neurosci Lett* 2001;304:157-60.
22. Buetler, T. M., Krauskopf, A. & Ruegg, U. T. Role of superoxide as a signaling molecule. *News Physiol Sci* 2004;19:120-3.
23. Huang WC, Chio CC, Chi KH, Wu HM, Lin WW. Superoxide anion-dependent Raf/MEK/ERK activation by peroxisome proliferator activated receptor gamma agonists 15-deoxy-delta(12,14)-prostaglandin J(2), ciglitazone, and GW1929. *Exp Cell Res* 2002;277:192-200.