

Cur2004-8, a synthetic curcumin derivative, extends lifespan and modulates age-related physiological changes in *Caenorhabditis elegans*

Bo-Kyoung Kim¹, Sung-A Kim¹, Sun-Mi Baek¹, Eun Young Lee^{2,3}, Eun Soo Lee⁴, Choon Hee Chung⁴, Chan Mug Ahn⁵, Sang-Kyu Park^{1,*}

¹Department of Medical Biotechnology, Soonchunhyang University, Asan, Chungnam, Republic of Korea;

²Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Cheonan, Chungnam, Republic of Korea;

³Institute of Tissue Regeneration, College of Medicine, Soonchunhyang University, Cheonan, Chungnam, Republic of Korea;

⁴Department of Internal Medicine, Wonju College of Medicine, Yonsei University, Wonju, Gangwon, Republic of Korea;

⁵Department of Basic Science, Wonju College of Medicine, Yonsei University, Wonju, Gangwon, Republic of Korea.

Summary

Curcumin, a compound found in Indian yellow curry, is known to possess various biological activities, including anti-oxidant, anti-inflammatory, and anti-cancer activities. Cur2004-8 is a synthetic curcumin derivative having symmetrical bis-alkynyl pyridines that shows a strong anti-angiogenic activity. In the present study, we examined the effect of dietary supplementation with Cur2004-8 on response to environmental stresses and aging using *Caenorhabditis elegans* as a model system. Dietary intervention with Cur2004-8 significantly increased resistance of *C. elegans* to oxidative stress. Its anti-oxidative-stress effect was greater than curcumin. However, response of *C. elegans* to heat stress or ultraviolet irradiation was not significantly affected by Cur2004-8. Next, we examined the effect of Cur2004-8 on aging. Cur2004-8 significantly extended both mean and maximum lifespan, accompanying a shift in time-course distribution of progeny production. Age-related decline in motility was also delayed by supplementation with Cur2004-8. In addition, Cur2004-8 prevented amyloid-beta-induced toxicity in Alzheimer's disease model animals which required a forkhead box (FOXO) transcription factor DAF-16. Dietary supplementation with Cur2004-8 also reversed the increase of mortality observed in worms treated with high-glucose-diet. These results suggest that Cur2004-8 has higher anti-oxidant and anti-aging activities than curcumin. It can be used for the development of novel anti-aging product.

Keywords: Curcumin, Cur2004-8, oxidative stress, aging, *Caenorhabditis elegans*

1. Introduction

Aging is one of the most complex biological phenomena. It exhibits various physiological and pathological changes in organisms. To understand aging process, many theories have been suggested. Among them, the most cited and studied one is the free radical theory introduced by Dr. Herman (1). According to this free

radical theory, when free radicals are accumulated with aging, they cause cellular damages and dysfunction of cellular biomolecules, eventually leading to organism's death. Major free radicals found in cells are reactive oxygen species (ROS) that can cause oxidative damages to DNA, protein, and lipid. A lot of studies have focused on role of anti-oxidant genes in aging. In *C. elegans*, although knockout of anti-oxidant genes could reduce resistance of *C. elegans* to oxidative stress and lifespan, over-expression of anti-oxidant genes failed to extend lifespan (2). Simultaneous over-expression of both Cu/Zn-superoxide dismutase and catalase significantly increased lifespan of *Drosophila melanogaster* (3). However, the longevity phenotype

*Address correspondence to:

Dr. Sang-Kyu Park, Department of Medical Biotechnology, Soonchunhyang University, 22 Soonchunhyang-ro, Asan, Chungnam 31538, Republic of Korea.

E-mail: skpark@sch.ac.kr

conferred by double transgenic animals was observed only in short-lived strain, not in long-lived strain of *Drosophila melanogaster* (4). Transgenic mice having additional copy of anti-oxidant genes showed no lifespan extension (5). Over-expression of human catalase with mitochondrial target signal in mice induced significant lifespan extension (6). The role of anti-oxidant genes in lifespan remains elusive.

Effects of dietary supplementation with various anti-oxidants on aging have been reported. Resveratrol, a polyphenol compound found in red wine, can extend lifespans of many experimental organisms. In *C. elegans*, treatment with resveratrol increased its lifespan to 18% and reduced its susceptibility to oxidative stress (7). The increase in lifespan induced by resveratrol is accompanied by reduced fecundity as a trade-off (8). The lifespan-extending effect of resveratrol was also observed in yeast and fruit fly (9,10). Polyphenol extracted from blueberry can increase resistance of *C. elegans* to heat stress, delay age-related decline of pharyngeal pumping, and confer 28% increase of its lifespan (11). Vitamin E has lifespan-extending effects in nematode and mice (12,13). Genomic transcriptional profiling study has revealed tissue-specific transcriptional biomarkers of aging (14). In addition, dietary supplementation with anti-oxidants such as lipoic acid, coenzyme Q10, lycopene, and tempol can markedly block age-related transcriptional changes in mice (14).

Curcumin is a bio-active ingredient found in turmeric, *Curcuma longa*. It has been used as a traditional therapeutic compound due to its anti-inflammatory, anti-oxidant, and chemo-preventive activities. In recent

years, curcumin has been shown to possess anti-cancer activities. It can reduce tumor formation and progression (15). Curcumin also shows protective effect against many chronological diseases, including cardiovascular and neurological diseases (16). A recent study has shown that curcumin can mediate lifespan extension and modulate many age-related biomarkers, including accumulation of lipofuscin, fertility, response to environmental stressors, and cellular ROS production in *C. elegans* (17). Curcumin can also inhibit amyloid beta (A β)-induced pathology in Alzheimer's disease (AD) model mice (18).

In the present study, we examined effect of curcumin derivative Cur2004-8 on aging using *C. elegans* as a model system. Cur2004-8, a synthetic curcumin derivative produced by Sonogashira reaction, has symmetrical bis-alkynyl pyridines connected by aromatic linker (Figure 1A). A previous study has shown that Cur2004-8 has stronger anti-angiogenic activity *in vitro* than curcumin (19). We compared the anti-stress effect of Cur2004-8 to that of curcumin and examined the anti-aging activity of Cur2004-8. Effect of dietary supplementation with Cur2004-8 on age-related diseases was also monitored. Results of this study support the free radical theory of aging and suggest that Cur2004-8 can be useful for the development of novel anti-aging therapeutics.

2. Materials and Methods

2.1. *C. elegans* strains and maintenance

Bristol N2 strain of *C. elegans* was used as a wild-type

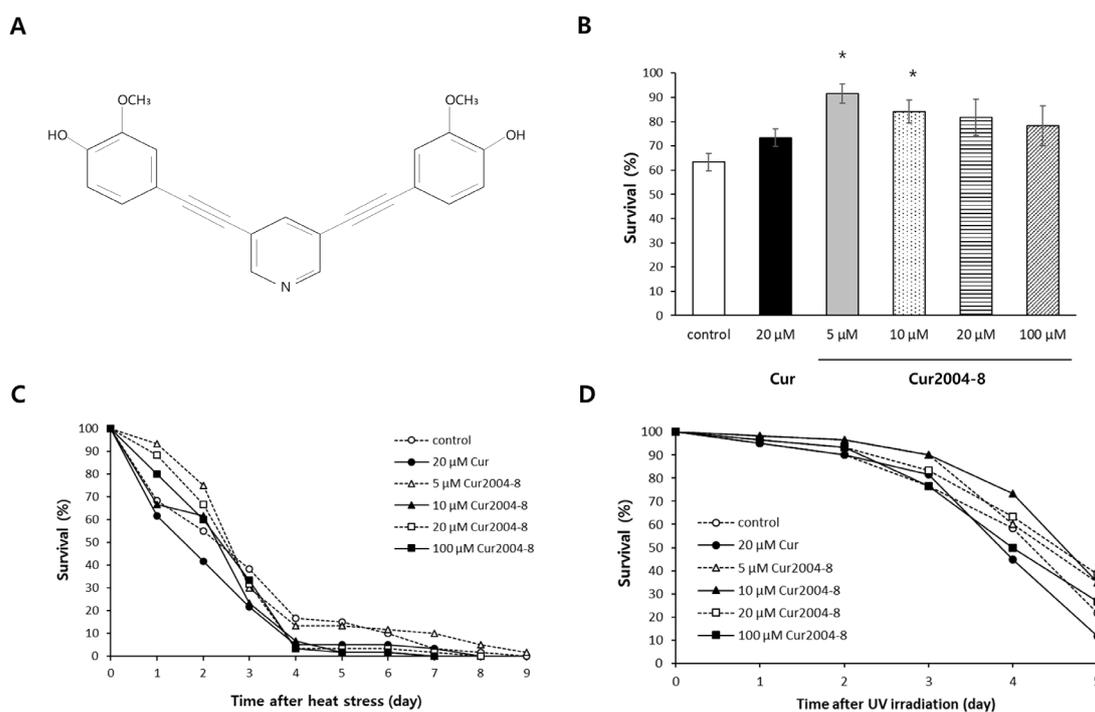


Figure 1. Effect of Cur2004-8 on response to environmental stressors. (A) Structure of Cur2004-8. Survival after oxidative stress **(B)**, heat stress **(C)**, or UV irradiation **(D)** was compared between untreated control and curcumin- or Cur2004-8-treated group. Cur, curcumin; *, significantly different from untreated control ($p < 0.05$).

control in all experiments. The CL4176 strain expressing muscle-specific human A β ₁₋₄₂ (*dvl527 [myo-3/A β ₁₋₄₂/let UTR, rol-6]*) was used for A β -induced toxicity assay. All strains were purchased from the *Caenorhabditis elegans* Genetics Center (CGC, Minneapolis/St. Paul, MN, USA). Worms were maintained on solid Nematode Growth Media (NGM) (25 mM NaCl, 1.7% agar, 2.5 mg/mL peptone, 50 mM KH₂PO₄ (pH 6.0), 5 μ g/mL cholesterol, 1 mM CaCl₂, and 1 mM MgSO₄) plates at 20°C. *Escherichia coli* OP50 was provided as food source.

2.2. Resistance to oxidative stress

For age-synchronization, five young adult worms obtained from 3-day-old mothers were transferred to a fresh NGM plate and permitted to lay eggs for 6 h. After removing these five adult worms from the plate, remaining eggs were incubated at 20°C for 3 days to hatch and grow. Three-day-old age-synchronized worms ($n = 30$) were transferred to fresh NGM plates treated with curcumin or Cur2004-8 and incubated at 20°C for 1 day. These worms were then placed under oxidative stress condition with 2 mM hydrogen peroxide (H₂O₂) (Sigma-Aldrich, St. Louis, MO, USA) in S-basal without cholesterol (5.85 g sodium chloride, 1 g potassium phosphate dibasic, and 6 g potassium phosphate monobasic for 1 L sterilized distilled-water) for 6 h in 96-well plates.

2.3. Response to heat stress

Three-day-old age-synchronized worms ($n = 60$) were transferred to fresh NGM plates containing curcumin or Cur2004-8. After adapting for 24 h, worms were placed in a 35°C incubator for 7 h to be exposed to heat stress. These worms were then transferred back to 20°C and incubated for 24 h. The survival of worms was recorded daily until all worms were dead.

2.4. Survival after ultraviolet (UV) irradiation

Sixty age-synchronized young adult worms were treated with curcumin or Cur2004-8 for 24 h at 20°C and irradiated with 20 J/cm²/min of UV for 1 min in a UV crosslinker (BLX-254, VILBER Lourmat Co., Torcy, France). The survival of worms on NGM plates containing curcumin or Cur2004-8 was then monitored daily until all worms were dead.

2.5. Measurement of lifespan

Three-day-old age-synchronized worms ($n = 60$) were transferred to NGM plates containing curcumin or Cur2004-8. Live and dead worms were scored daily. Worms lost, killed, or having internal hatching were excluded. During the assay, worms were transferred

to fresh NGM plates every day during gravid period and every 2-3 days after gravid period. 5-Fluoro-2'-deoxyuridine (Sigma-Aldrich, St. Louis, MO, USA) (12.5 mg/L) was added to NGM plates to prevent internal hatching.

2.6. Assessment of fertility

Five L4/young adult worms were placed on a fresh NGM plate containing curcumin or Cur2004-8 at 20°C for 5 h to lay eggs. After removing all adult worms from the plate, eggs were maintained at 20°C for 2 days. Ten randomly selected worms were transferred individually to fresh NGM plates treated with curcumin or Cur2004-8 every day until worms no longer produced eggs. Hatched progeny from an individual worm on each day were counted after incubation at 20°C for 48 h.

2.7. Measurement of motility

Age-synchronized worms ($n = 100$) were grown on NGM plates containing curcumin or Cur2004-8. Change of motility was measured on 15 and 20 days after egg-hatching. Each worm was classified into one of three groups: 1) Phase 1, a worm that could move spontaneously without any mechanical stimuli; 2) Phase 2, a worm that moved only when mechanical stimuli were given; and 3) Phase 3, a worm that could only move its head part with mechanical stimuli.

2.8. A β -induced toxicity

Age-synchronized young adult CL4176 worms ($n = 30$) were permitted to lay eggs on NGM plates containing curcumin or Cur2004-8 for 2 h at 15°C. After removing all adult worms, the progeny was incubated at 15°C for 24 h. Randomly selected worms ($n = 60$) were then incubated for 24 h at 25°C to induce the expression of human A β transgene in muscle. The number of paralyzed worms was recorded every hour after A β induction.

2.9. RNA interference (RNAi)

To repress the expression of *daf-16*, we used *daf-16* RNAi clone obtained from the Ahringer RNAi library (20). Induction of *daf-16* double-stranded RNA was done by adding 0.4 mM isopropyl- β -D-thiogalactoside (IPTG, Sigma-Aldrich, St. Louis, MO, USA) to culture media after OD₆₀₀ reached 0.4. After culturing for additional 4 h, bacteria were used as food source for RNAi knockdown. RNAi clone carrying empty vector (EV) was used as a negative control.

2.10. Lifespan with high-glucose diet (HGD)

Sixty age-synchronized worms were randomly selected from 3-day-old young adult worms. These worms were

then transferred to a fresh NGM plate containing both OP50 and high-glucose (100 μ L of 40 mM glucose). The survival of worms was recorded daily until all worms were dead.

2.11. Statistical analysis

For comparison of resistance to oxidative stress and fertility between untreated control and curcumin- or Cur2004-8-treated group, we used the standard two-tailed Student's *t*-test. For statistical analysis of survival after heat stress or UV irradiation, lifespan, paralysis assay, and survival under HGD, we employed the log-rank test (21). The log-rank test is a non-parametric Mantel-Cox test widely used for statistical comparison between two survival curves. A *p*-value lower than 0.05 was considered as statistically significant.

3. Results

3.1. Cur2004-8 specifically increases resistance of *C. elegans* to oxidative stress

In order to examine the effect of Cur2004-8 on response of *C. elegans* to environmental stressors, survival of

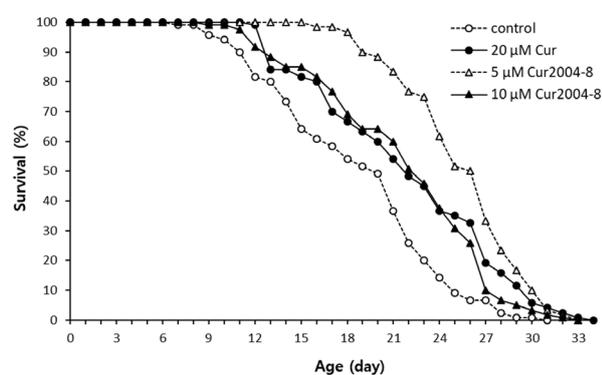


Figure 2. Lifespan extension by supplementation with Cur2004-8. Effect of dietary supplementation with curcumin or Cur2004-8 on lifespan of *C. elegans* was examined using age-synchronized animals ($n = 60$). Cur, curcumin.

worms was monitored after oxidative stress, heat stress, or UV irradiation. We compared the effect of different concentration of Cur2004-8 with that of 20 μ M of curcumin reported to be able to increase resistance of *C. elegans* to oxidative stress and its lifespan (19). Resistance to oxidative stress was significantly increased by supplementation with Cur2004-8 (Figure 1B). Mean survival rate of wild-type control was $63.3 \pm 3.60\%$ (mean of three replicates \pm SE) after 6 h of oxidative stress. Survival rate for the group supplemented with 20 μ M of curcumin was $73.3 \pm 3.60\%$ ($p = 0.097$), which was slightly increased compared to that of the wild-type control after 6 h of oxidative stress. However, mean survival rates for groups supplemented with 5 and 10 μ M of Cur2004-8 were significantly increased to $91.7 \pm 3.97\%$ ($p = 0.002$) and $84.2 \pm 4.79\%$ ($p = 0.014$), respectively. Although concentration of Cur2004-8 higher than 10 μ M increased the survival rate, the survival rate was not significantly different from that of the wild-type control ($p > 0.05$). However, there was no significant difference in response to heat stress or UV irradiation between control and Cur2004-8-treated groups (Figures 1C and 1D). These findings suggest that Cur2004-8 is more effective than curcumin in increasing resistance of *C. elegans* to oxidative stress and that its effect is specific for oxidative stress.

3.2. Supplementation with Cur2004-8 significantly extends lifespan of *C. elegans*

The free radical theory of aging emphasizes the role of oxidative stress in determination of organism's lifespan (1). Having observed anti-oxidative-stress effect of Cur2004-8, we next examined its effect on lifespan. Since 5 and 10 μ M of Cur2004-8 showed a significant increase in resistance to oxidative stress, we chose those two concentrations for the following experiments. Dietary supplementation with Cur2004-8 significantly extended both mean and maximum lifespan (Figure 2). Mean lifespan of untreated control (20.1 days) was increased to 24.6 days after supplementation with 5 μ M Cur2004-8 ($p < 0.001$). It was increased to 23.5

Table 1. Effect of Cur2004-8 on lifespan of *C. elegans*

Items	Mean lifespan (d)	Maximum lifespan (d)	<i>p</i> -value ^a	% effect ^b
1 st experiment				
control	20.1	30		
20 μ M Cur	25.4	33	< 0.001	26.6
5 μ M Cur2004-8	24.6	32	< 0.001	22.6
10 μ M Cur2004-8	23.5	32	< 0.001	17.4
2 nd experiment				
control	15.5	27		
20 μ M Cur	16.6	25	0.791	7.6
5 μ M Cur2004-8	24.6	32	0.001	59.1
10 μ M Cur2004-8	18.1	28	0.051	16.9

^a*p*-value was calculated using the log-rank test by comparing the survival of control group to that of Cur- or Cur2004-8-treated group. ^b % effects were calculated by $(C-T)/C \times 100$, where *T* is the mean lifespan of curcumin- or Cur2004-8-treated group and *C* is the mean lifespan of control group. Cur, curcumin.

days after supplementation with 10 μM Cur2004-8 ($p < 0.001$). Mean lifespans were increased 22.6 and 17.4% by 5 and 10 μM Cur2004-8, respectively. Maximum lifespan was also increased from 30 days to 32 days after treatment with Cur2004-8. An independent replicative experiment showed a positive effect of Cur2004-8 on lifespan (Table 1). The percent effect on mean lifespan was 59.1% with 5 μM Cur2004-8 ($p = 0.001$) while it was only 16.9% with 10 μM Cur2004-8 ($p = 0.051$). Unlike consistent lifespan-extending effect of Cur2004-8, 20 μM curcumin showed a significant lifespan-extending effect in the first experiment but failed to induce lifespan extension in the second experiment (Table 1). Our results indicate that Cur2004-8 can confer a longevity phenotype and that its lifespan-extending activity is greater than curcumin.

3.3. Cur2004-8 modulates reproduction of adult worms

Previous studies have reported that many lifespan-extending genetic or dietary interventions are

accompanied by reduced fertility as a trade-off (9,22,23). In the present study, dietary supplementation with Cur2004-8 did not affect total number of progeny produced during the gravid period (Figure 3A). There was a slight decrease in total number of progeny compared to untreated control, although the difference was not statistically significant ($p > 0.05$). However, time-course distribution of progeny produced was significantly modulated by curcumin or Cur2004-8 (Figure 3B). At 3 days after hatching, 89.7 ± 9.73 (mean of 10 individual worms \pm SEM) progeny were produced by the untreated control. The number of progeny produced was decreased by curcumin or Cur2004-8: 68.0 ± 3.25 ($p = 0.066$) for 20 μM curcumin-treated worms, 67.2 ± 2.49 ($p = 0.040$) for 5 μM Cur2004-8-treated worms, and 70.2 ± 3.74 ($p = 0.086$) for 10 μM Cur2004-8-treated worms. Statistically significant decreases in the number of progeny produced were detected in all curcumin- and Cur2004-8-treated groups on 4th day after hatching. The number of progeny was reduced from 122.4 ± 6.74 in the untreated control

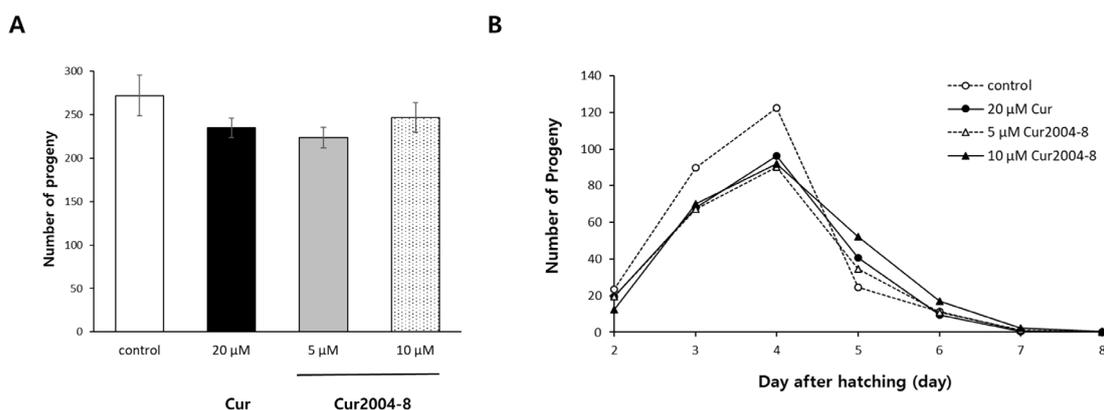


Figure 3. Effect of Cur2004-8 on reproduction. (A) Total number of progeny produced during the gravid period was counted ($n = 10$). Error bar indicates standard error of mean. (B) Time-course distribution of progeny in untreated control, curcumin-treated, and Cur2004-8-treated group. Cur, curcumin.

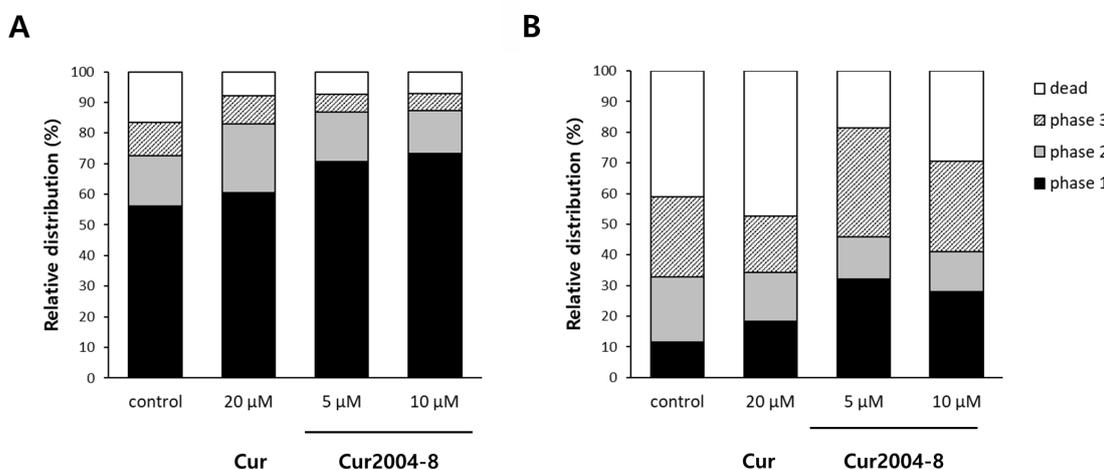


Figure 4. Delayed decline of motility by Cur2004-8. Effect of curcumin or Cur2004-8 on motility was determined in 15-day-old (A) and 20-day-old animals (B) ($n = 100$). Worms were classified into three phases according to their locomotive activity: Phase 1, a worm that could move spontaneously without any mechanical stimuli; Phase 2, a worm that moved only when mechanical stimuli were given; and Phase 3, a worm that could only move its head part with mechanical stimuli. Cur, curcumin.

to 96.4 ± 2.46 ($p = 0.005$), 90.3 ± 2.82 ($p < 0.001$), and 92.1 ± 2.59 ($p = 0.001$) in 20 μM curcumin-, 5 μM Cur2004-8-, and 10 μM Cur2004-8-treated group, respectively. However, the number of progeny produced in curcumin- or Cur2004-8-treated group was higher than that in the untreated control on 5 days after hatching. Numbers of progeny in worms treated with 20 μM curcumin, 5 μM Cur2004-8, and 10 μM Cur2004-8 were 40.5 ± 2.69 ($p = 0.016$), 34.5 ± 3.65 ($p = 0.108$), and 52.0 ± 5.02 ($p = 0.001$), respectively, compared to the number of progeny produced in the untreated control (24.4 ± 3.53) (Figure 3B). These data suggest that dietary supplementation with curcumin or Cur2004-8 does not affect the reproducibility of *C. elegans*, although it can induce a significant shift in time-course distribution of progeny during the gravid period.

3.4. Age-related decline of motility is delayed by Cur2004-8

Next, we determined the effect of Cur2004-8 on change of motility with time to elucidate its role in muscle aging. At 15 days after hatching, 56% of the untreated control worms and 61% of worms treated with 20 μM curcumin showed spontaneous motility without any mechanical stimuli (phase 1) while more worms (71% with 5 μM Cur2004-8 and 73% with 10 μM Cur2004-8) were classified as phase 1 (Figure 4A). Bigger difference in motility was observed on 20th day. In the untreated control, only 11% of worms could move spontaneously and 18% of worms showed 'phase 1' phenotype in 20 μM curcumin-treated group. However, percentages of worms categorized as 'phase 1' were increased to 32% and 29% by supplementation with 5 and 10 μM Cur2004-8, respectively (Figure 4B). In addition, percentages of dead worms showing no motility was reduced by Cur2004-8. In untreated control and 20 μM curcumin-treated group on day 20, 41% and 47% of worms were not moving at all, respectively. Percentages of worms showing no motility were

reduced to 19% and 30% by dietary supplementation with 5 and 10 μM Cur2004-8, respectively (Figure 4B). These results suggest that Cur2004-8 could slow down age-related dysfunction of muscle in *C. elegans*.

3.5. Cur2004-8 suppresses A β -induced toxicity via DAF-16

To examine the effect of Cur2004-8 on age-related disease, we employed a transgenic AD model expressing human A β . Accumulation of transgenic human A β in muscle is known to induce paralysis and lead to death (24). Mean survival time of the untreated control was 4.1 h. Dietary supplementation with Cur2004-8 significantly delayed paralysis. Cur2004-8 at 5 and 10 μM increased mean survival time to 6.6 h ($p < 0.001$) and 5.7 h ($p = 0.003$), respectively (Figure 5A). Treatment with 20 μM curcumin also reduced A β -induced toxicity (mean survival time 5.8 days, $p = 0.004$). Thus, 20 μM curcumin, 5 μM Cur2004-8, and 10 μM Cur2004-8 increased mean survival time by 42.6%, 61.1%, and 41.0%, respectively (Table 2). A previous study has reported that DAF-16 is required for the inhibition of A β -induced toxicity (25). Interestingly, increased survival after A β induction by Cur2004-8 completely disappeared when the expression of *daf-16* was knocked down (Figure 5B). Thus, we conclude that Cur2004-8 can reduce A β -induced toxicity more efficiently than curcumin and that it requires DAF-16 for its preventive effect.

3.6. Decreased survival with HGD is recovered by supplementation with Cur2004-8

Since HGD can reduce survival of *C. elegans*, it can be used as a nutritional model of diabetes mellitus (26). In the present study, HGD reduced the lifespan of *C. elegans* compared to the untreated control. Mean lifespan was significantly decreased from 16.7 days in the untreated control to 13.5 days in the HGD group ($p = 0.001$) (Figure 6). However, decreased lifespan by

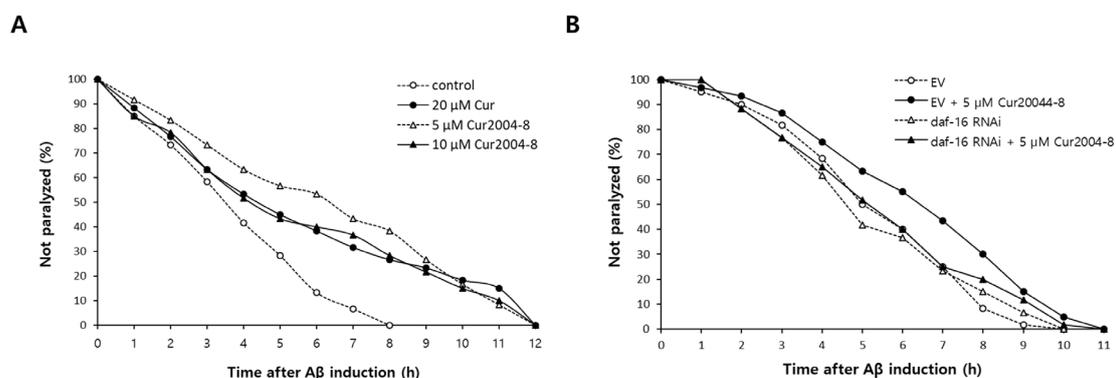


Figure 5. Reduced susceptibility to A β -induced toxicity by Cur2004-8. (A) Paralysis caused by muscle-specific expression of human A β was monitored. **(B)** Effect of *daf-16* knockdown on protective effect of Cur2004-8 against A β -induced paralysis was determined using RNAi. Cur, curcumin; EV, empty vector.

Table 2. Effect of Cur2004-8 on A β -induced toxicity in *C. elegans*

Items	Time when 50% of worms were paralyzed (h)	<i>p</i> -value ^a	% effect ^b
1 st experiment			
control	4.1		
20 μ M Cur	5.8	< 0.01	42.6
5 μ M Cur2004-8	6.6	< 0.001	61.1
10 μ M Cur2004-8	5.7	< 0.01	41.0
2 nd experiment			
control	5.1		
20 μ M Cur	6.2	< 0.05	23.0
5 μ M Cur2004-8	6.2	< 0.05	22.0
10 μ M Cur2004-8	6.0	0.075	19.1

^a*p*-value was calculated using the log-rank test by comparing the survival of control group to that of Cur- or Cur2004-8-treated group. ^b % effects were calculated by $(C-T)/C*100$, where *T* is the mean lifespan of curcumin- or Cur2004-8-treated group and *C* is the mean lifespan of control group. Cur, curcumin.

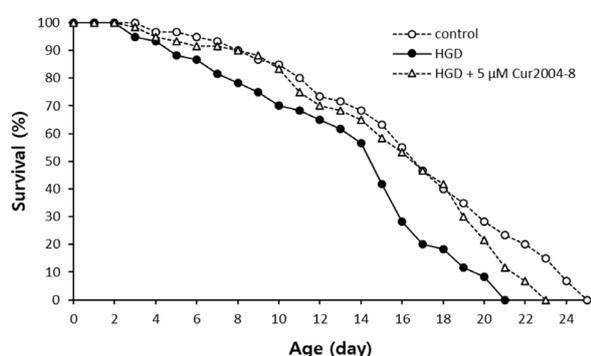


Figure 6. Effect of Cur2004-8 on HGD-induced toxicity. Survival of animals was compared between untreated control and worms fed with HGD, and preventive effect of Cur2004-8 on decrease in survival by HGD was examined. Cur, curcumin; HGD, high-glucose diet.

HGD was markedly restored by supplementation with Cur2004-8. Mean lifespan was increased up to 15.8 days for HGD-worms treated with 5 μ M Cur2004-8, which was 71.9% increase compared to worms treated with HGD alone ($p = 0.009$). Maximum lifespan was also decreased by HGD and restored for worms treated with both HGD and Cur2004-8 (Figure 6). These results suggest that Cur2004-8 has a preventive effect on HGD-induced toxicity in addition to its effect on A β -induced toxicity in *C. elegans*.

4. Discussion

An increasing number of aging studies have been focused on the identification of anti-aging dietary interventions. The most effective intervention identified so far is dietary restriction (DR) which retards age-related physiological changes and extends lifespan in many model organisms (27). However, due to difficulty in practicing DR in daily life, people are searching for nutritional interventions that can delay aging process without DR. Anti-oxidants are believed to be the most promising compounds based on the free radical theory. Curcumin is a well-known anti-oxidant with anti-aging effect. Dietary supplementation with

curcumin can reduce intracellular ROS and increase resistance to oxidative stress (28). Expression levels of stress-responsive genes are significantly induced by curcumin (28). Curcumin can modulate activities of anti-oxidant enzymes and reduced protein oxidation (29,30). Lifespan extension after supplementation with curcumin has also been reported with 20 μ M and there was no significant difference in lifespan with higher dose (200 μ M) of curcumin (17). Results of the present study showed that Cur2004-8 as a synthetic curcumin derivative had higher anti-oxidant and lifespan-extending effects than free curcumin. Recent studies have reported that trimethoxystilbene derivative of resveratrol showed better bioavailability and elevated anti-cancer activity than resveratrol (31). Resveratrol-maltol hybrids inhibited aggregation of A β (32). In addition, resveratrol peptidyl derivatives exhibited strong anti-oxidant and anti-cancer activities (33). Further studies revealing the molecular basis of improved activities of Cur2004-8 and anti-aging effect in higher organisms should be followed in the near future.

Many studies have reported that there is a trade-off for lifespan extension by genetic or dietary intervention. Longevity phenotype observed in *daf-2* mutants is accompanied by delayed development and reduced reproduction (22). The *age-1* mutation also exhibited reduced fertility as a trade-off for long lifespan (23). Resveratrol resulted in early onset of egg-laying and extended the gravid period with increased lifespan (8). In case of blueberry polyphenols, long-lived worms showed delayed decline of pharyngeal pumping rate with aging (11). We observed a shift in time-course distribution of progeny in worms supplemented with Cur2004-8, suggesting that Cur2004-8 might induce allocation of cellular limited resource from reproduction toward survival and maintenance. The disposable soma theory of aging emphasizing role of fitness cost paid for lifespan extension supports our findings (34).

Muscle cells are among the most energy-demanding cells and aging muscle is characterized by loss of mass and reduced strength (35). A previous study has

shown that transgenic mice carrying additional copy of mitochondrial catalase have delayed dysfunction of mitochondria (6). Dietary supplementation with antioxidants including silymarin and phycoerythrin can enhance locomotion rate and increase lifespan (36,37). Lifespan-extending selenocysteine can also significantly delay age-related decline of motility in *C. elegans* (38). Here, we observed enhanced motility in aged animals treated with Cur2004-8. Our findings suggest that Cur2004-8 can retard muscle aging possibly through its anti-oxidant activity.

Effects of curcumin on age-related diseases have been reported. Treatment with tetrahydrocurcumin can reduce ROS production caused by A β in rat hippocampal cells (39). α -Synuclein-induced cell death is significantly inhibited by curcumin (40). In AD model mice, curcumin reduced lipid peroxidation and restored motor function (41). Encapsulated curcumin nanoparticle rescued neuromotor deficits observed in Huntington's disease model animals (42). Our results showed that Cur2004-8 could ameliorate A β -induced toxicity in *C. elegans* genetic model of AD and that its effect required DAF-16. Additionally, dietary supplementation with Cur2004-8 completely blocked the toxic effect of HGD which was used as a nutritional model of diabetes mellitus.

In conclusion, Cur2004-8 has anti-oxidant and anti-aging activities and protective activity against toxicities involved in age-related diseases. Cur2004-8 shows improved bioactivities compared to free curcumin. The effect of 5 μ M Cur2004-8 was higher than that of 20 μ M free curcumin in oxidative stress, lifespan, motility, and A β -induced toxicity assays. Further studies identifying downstream molecular targets of Cur2004-8 and determining its *in vivo* effect in higher organisms such as mice are necessary.

Acknowledgements

This work was supported by the Soonchunhyang University Research Fund and the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2018R1D1A1B07043414).

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(Received July 16, 2019; Revised August 20, 2019; Accepted August 21, 2019)