SUPPLEMENTATION OF S-ALLYL CYSTEINE IMPROVES HEALTH SPAN
IN Caenorhabditis elegans

A SUPLEMENTAÇÃO DE S-ALIL CISTEÍNA MELHORA A DURAÇÃO DA SAÚDE EM Caenorhabditis elegans

Jun-Sung KIM1; Sang-Kyu PARK2

1. Graduate student, Department of Medical Sciences, Soonchunhyang University, Asan, Chungnam, Korea; 2. Associate
Professor, Department of Medical Biotechnology, Soonchunhyang University, Asan, Chungnam, Korea. skpark@sch.ac.kr

ABSTRACT: Previous studies show that nutritional interventions with anti-oxidants have various health-promoting effects in several model organisms. Here, we examine the effects of S-allyl cysteine on resistance to environmental stressors and age-related physiologic changes using C. elegans as a model system. S-allyl cysteine is a modified amino acid found in aged garlic extracts and known to have strong anti-oxidant activity. The survival of worms under oxidative-stress conditions significantly increased with supplementation of S-allyl cysteine. In addition, pre-treatment of S-allyl cysteine significantly increased resistance to both heat stress and ultraviolet irradiation. However, lifespan was not affected by S-allyl cysteine treatment. We also examined the effect of S-allyl cysteine on motility of C. elegans and found that S-allyl cysteine can retard the age-related decline of muscle tissue locomotive activity. S-allyl cysteine also significantly suppressed amyloid β-induced paralysis in Alzheimer’s disease model animals. Taken together, our study indicates that dietary supplementation of S-allyl cysteine can improve health span and suggests that S-allyl cysteine can be used to develop novel health-promoting pharmaceuticals.


INTRODUCTION

S-allyl cysteine (SAC) is an organosulfur compound found in aged garlic. In addition to SAC, aged garlic extracts contain allin, cycloallin, S-methyl cysteine, β-chlorogenin, and among others (ALLISON et al., 2006). Aged garlic extracts possess many pharmaco-therapeutic properties, including anti-oxidant, anti-allergic, anti-cancer, neuro-protective, and hepatoprotective activities (COLIN-GONZALEZ et al., 2015; MORIHARA et al., 2006). SAC is the most abundant compound in aged garlic and has strong anti-oxidant activity (COLIN-GONZALEZ et al., 2015). At the cellular level, reactive oxygen species (ROS), including superoxide radical and hydrogen peroxide (H₂O₂), are reduced by SAC treatment (IDE; LAU, 2001; MALDONADO et al., 2003). SAC can scavenge superoxide radical and reduce hydrogen peroxide production (COLIN-GONZALEZ et al., 2012; MEDINA-CAMPO et al., 2007). A recent study suggests that the underlying mechanisms of SAC anti-oxidant activity involves the induction of Nrf2 protein, a transcription factor modulating expressions of many anti-oxidant genes, both in vitro and in vivo in mice (SHI et al., 2015). In C. elegans, the effect of SAC is dependent on SKN-1, a worm homolog of Nrf-2, and independent on DAF-16 (OGAWA et al., 2016). Oxidative stress caused by ROS is considered one of major causal factors of many neurodegenerative diseases. In rats, supplementation of SAC reduces amyloid beta (Aβ)-induced oxidative damage in the brain and improves cognitive function (PEREZ-SEVERIANO et al., 2004). The neuroprotective effect of SAC is further supported by a decrease in Aβ-induced apoptosis in hippocampal neurons (PENG et al., 2002).

A number of studies have shown that dietary intervention with anti-oxidants has health-promoting effects in various model organisms. Supplementation of resveratrol, a polyphenol compound contained in red wine, gives various health benefits, such as mitigation of neurodegeneration, carcinogenesis and atherosclerosis (FERGUSON, 2001; JANG et al., 1997). Cognitive impairment observed in aged animals is prevented by vitamin E supplementation in rats and dogs (FUKUI et al., 2002; MILGRAM et al., 2002). Curcumin or green tea polyphenol metabolites are effective in preventing age-associated disorders, including cancer and atherosclerosis in mice (KITANI et al., 2004). The increased oxidant production and oxidative DNA damage are opposed by α-lipoic acid supplementation (SUH et al., 2001). Coenzyme Q₁₀ promotes the recovery of heart tissue after aerobic stress and the contractile function after cardiac surgery (ROSENFELDT et al., 2002).

Received: 12/01/16
Accepted: 05/11/16

Supplementation of S-allyl cysteine...

KIM, J-S.; PARK; S-K.

resistance to oxidative, heat, and ultraviolet (UV) stresses (OH et al., 2015).

*Caenorhabditis elegans* (*C. elegans*) is a widely used animal model for the screening of compounds or natural extracts expected to have health-promoting properties due to its relative short life cycle and lifespan, large brood size, and most importantly, high homology with the human genome. Comparative proteomic study reveals that at least 83% of *C. elegans* genes has human homologs (Lai et al., 2000). In the present study, we intend to reveal the effects of SAC supplementation on responses to environmental stressors and age-related physiological changes, *in vivo*, using *C. elegans* as a model system.

**MATERIALS AND METHODS**

**Worm strains and maintenance**

The N2 CGCb strain purchased from the *C. elegans* Genetics Center (CGC, Minneapolis, USA) was used as wild-type control. The Alzheimer’s disease model strain expressing human Aβ_{1-42} in muscle, CL4176 (dvl27 [myo-3/Aβ1-42/let UTR, rol-6]), was also purchased from CGC (Minneapolis, USA). Nematode growth media (NGM) containing 25 mM NaCl, 1.7% agar, 2.5 mg/ml peptone, 5 μg/mL cholesterol, 1 mM CaCl₂, 1 mM MgSO₄, and 50 mM KH₂PO₄ (pH6.0), was used as growth media. All experiments were conducted at 20°C, unless the specific temperature shift was mentioned. *Escherichia coli* OP50 was added to each NGM plate as a food source.

**Survival under oxidative stress**

Five L4/young adult worms were transferred to a fresh NGM plate and permitted to lay eggs for 5 h at 20°C. After removing the five adult worms, the progeny were grown at 20°C for 3 days. Age-synchronized worms were treated with different concentrations of SAC (0, 1, 5, 10 μM) for 24 h. Then, the worms were exposed to 2 mM H₂O₂ 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). The survival of worms under oxidative-stress conditions was monitored at 2, 4, and 6 h. A worm not responding to any mechanical stimuli was considered dead. Three independent repetitive experiments were performed. Statistical significance was measured using the standard two-tailed Student’s t-test. A p-value lower than 0.05 was considered significant.

**Response to heat stress**

Sixty age-synchronized worms were treated with different concentrations of SAC on NGM plates for 24 h as previously mentioned and heat shock was administered to the worms by placing the plates in a 35°C incubator for 10 h. Then, the worms were transferred back to 20°C and the number of dead worms was counted after 24 h. We replicated the independent experiments three times.

**Resistance to UV irradiation**

Age-synchronized worms were cultured in NGM plates containing 5 μM SAC for 24 h and exposed to 20 J/cm²/min UV for 1 min in a 254 nm-UV crosslinker (BLX-254, VILBER Lourmat Co., Torcy, France). After UV irradiation, the plates were transferred back to the 20°C incubator. Living and dead worms were scored every hour until all worms were dead. The experiments were repeated twice. We employed the log-rank test to analyze the data. The log-rank test is a non-parametric Mantel-Cox test, widely used to compare two time-course survival curves (PETO; PETO, 1972).

**Lifespan assay**

Sixty age-synchronized 3-day-old worms were transferred to fresh NGM plates containing 5 μM SAC and 5-fluoro-2’-deoxyuridine to prevent internal hatching. Thereafter, worms were transferred to fresh NGM plates with the 5 μM SAC and 5-fluoro-2’-deoxyuridine every other day until all worms were dead. The number of living and dead worms was scored every day. Three independent replicative experiments were performed. Statistical analysis was performed using the log-rank test (PETO; PETO, 1972).

**Locomotion assay**

The effects of SAC on age-related motility change was examined in age-synchronized worms (n=100). Each worm’s response to mechanical stimuli was monitored and recorded at 5, 10, 15, and 20 days after hatching. A worm that moved spontaneously without mechanical stimuli was grouped as “level 1”. Worms that moved their whole body or only head after worm picker stimulation were recorded as “level 2” or “level 3”, respectively. Dead worms were considered animals not responding to any mechanical stimuli. The experiments were repeated twice.

**Paralysis assay using human Aβ transgene**

Age-synchronized young adult worms laid eggs for 2 h at 15°C. Then, adult worms were
removed from the plates. The plates containing only eggs were incubated at 25°C to induce the human \( \text{A} \beta \) transgene. After 18-20 h of induction, the number of worms paralyzed were scored every hour until all worms were paralyzed. We repeated the experiments three times.

RESULTS AND DISCUSSION

SAC increases resistance to oxidative stress

In order to evaluate the effects of SAC on environmental stressors response, we first examined the SAC supplementation on resistance to oxidative stress. Oxidative stress induced by \( \text{H}_2\text{O}_2 \), an innate ROS produced by cellular metabolism, resulted in decreased survival in untreated wild-type control (Fig. 1). However, worms pre-treated with SAC reduced susceptibility to oxidative stress. Among three different concentrations tested, 5 \( \mu \text{M} \) of SAC significantly increased resistance to oxidative stress. After 2 h under oxidative-stress conditions, 45.6 ± 17.88% (mean of three independent experiments ± SEM) of worms survived in the wild-type control group and 86.7 ± 5.09% of worms were still alive in SAC-treated worms. The percent survival decreased to 12.2 ± 4.84% in the control, whereas 33.3 ± 12.02% of SAC-treated worms survived after 4 h. The effect of SAC on survival under oxidative-stress conditions increased from 2.2 ± 2.22% in control to 23.3 ± 1.92% after 6 h (\( p = 0.002 \)) (Fig. 1). The supplementation with 1 or 10 \( \mu \text{M} \) of SAC failed to show a significant increase in resistance to oxidative stress in all time points tested (\( p > 0.05 \)). These findings indicate that dietary supplementation with 5 \( \mu \text{M} \) SAC can confer increased resistance to oxidative stress in \( C. \text{elegans} \). Nutritional intervention studies using anti-oxidants have shown that there is a consistent correlation between dietary supplementation of anti-oxidants and response to oxidative stress. Polyphenol compounds found in red wine and green tea increase resistance to oxidative stress (KITANI et al., 2004; WOOD et al., 2004). Extracts from \( Acanthopanax \text{ sessiliflorus} \) stem shows a suppressive effect on oxidative DNA damage and increased survival under oxidative-stress conditions (PARK et al., 2014). Interestingly, NAC, the other cysteine derivative, has strong anti-oxidant activity \( \text{in vivo} \) and induces the expression of anti-oxidant gene (OH et al., 2015). Therefore, further studies should focus on revealing underlying cellular mechanisms involved in increased resistance to oxidative stress by these cysteine derivatives.

Figure 1. Increased resistance to oxidative stress by SAC. Oxidative stress was induced by treatment of 2 mM \( \text{H}_2\text{O}_2 \). The effect of different SAC concentrations on survival under oxidative-stress conditions was monitored at 2, 4, and 6 h after \( \text{H}_2\text{O}_2 \) addition. Pre-treatment of 5 \( \mu \text{M} \) SAC significantly increased survival of worms after 6 h of oxidative stress. The values are mean of three independent experiments. Asterisk indicates a statistically significant difference between control and SAC-treated worms.
Effects of SAC on response to heat stress and UV irradiation

Having observed increased survival under oxidative-stress conditions by SAC, we next determined the effect of SAC supplementation on other environmental stressors responses, including heat stress and UV irradiation. In the untreated wild-type control group, 59.0 ± 3.09% of worms survived after 10 h of heat shock (Fig. 2). Treatment of different concentrations of SAC before heat shock increased survival after heat stress. As observed in response to oxidative stress, 5 µM of SAC was the most effective concentration among three concentrations tested. The survival of worms treated with 5 µM of SAC was increased up to 82.2 ± 4.09% (p = 0.010) (Fig. 2). The percent survival observed in worms supplemented with 1 and 10 µM of SAC was also slightly increased up to 72.3 ± 5.30 and 79.3 ± 7.89%, respectively, but failed to show a statistically significant difference (p > 0.05). Based on previous results, we examined the effects of SAC on UV irradiation with 5 µM of SAC. Supplementation of SAC significantly increased survival after UV irradiation (p < 0.001). Mean survival time was 4.7 and 6.2 h in the wild-type control and SAC-treated worms, respectively (Fig. 3A). There was a 31.8% increase in mean survival time by SAC supplementation. The curve area was increased from 321.9 to 472.0 by SAC supplementation. Independent repeated experiments also showed significant increases in resistance to UV irradiation. There was an 11.2% increase in mean survival time and curve area was increased from 296.6 to 346.7 by SAC supplementation (p = 0.033) (Fig. 3B). Many studies have shown that increased resistance to oxidative stress accompanies increased survival under other environmental stressors. Worms grown in media prepared with electrolyzed-reduced water have increased resistance to oxidative stress and survive longer under heat stress or UV irradiation (PARK et al., 2012; PARK; PARK, 2013). Mutations in age-1 confer reduced susceptibility to both oxidative and heat stress (LARSEN, 1993). The supplementation of NAC modulates response to heat stress, in addition to oxidative stress. Increased survival after heat shock and induction of the hsp-16.2 gene is observed with NAC treatment (OH et al., 2015). Similar to our observation with SAC, worms with NAC supplementation also survive longer after UV irradiation (OH et al., 2015). Taken together, we conclude that SAC has a strong bioactivity modulating response to various environmental stressors.

Figure 2. Effects of SAC on thermotolerance. Survival of worms after 10 h of heat shock was compared between the wild-type control and SAC-treated worms. Among different concentrations of SAC tested, only 5 µM SAC conferred extended survival after heat shock. The values are mean of three independent experiments. Asterisk indicates a statistically significant difference between the control and SAC-treated worms.
Supplementation of S-allyl cysteine... KIM, J-S.; PARK; S-K.

Figure 3. Extended survival after UV irradiation by SAC. The effect of 5 μM SAC, which showed an increased resistance to oxidative and heat stress, on response to UV stress was determined twice (A and B). Both mean and maximum survival time was significantly extended by SAC treatment (p < 0.05).

SAC treatment has no effect on lifespan

The free radical theory of aging suggests that oxidative damage caused by free radicals is a major causal factor of normal aging (HERMAN, 1956). The most abundant free radicals found in cells are ROS produced as byproducts of cellular metabolism. Since we observed increased resistance to oxidative stress by the supplementation of SAC, we asked whether dietary intervention with SAC could extend C. elegans’ lifespan. As shown in Fig. 4, there is no significant difference in both mean and maximum lifespan between the untreated wild-type control and SAC-treated worms. Three independent experiments failed to show a significant effect on lifespan by SAC (data not shown). On the contrary, a recent study reports that higher concentrations of SAC, 10 and 100 μM, can extend mean lifespan up to 17.0 and 15.6%, respectively (OGAWA et al., 2016). The effect of dietary anti-oxidants on lifespan is very complicated in various model organisms. For example, resveratrol or myricetin significantly extends the lifespan of C. elegans, while SOD/catalase mimetics show a controversial effect on lifespan (BAYNE; SOHAL, 2002; GRUNZ et al., 2012; WOOD et al., 2004). In mice, the supplementation of vitamin E or curcumin results in a longevity phenotype, whereas dietary intervention with coenzyme Q10 or α-lipoic acid has no effect on lifespan (KITANI et al., 2004; LEE et al., 2002; NAVARRO et al., 2005). The effect of dietary anti-oxidants on lifespan seems to be variable depending on anti-oxidant molecules and model organisms, which causes controversy in authenticity of the free radical theory of aging. Interestingly, a previous study reveals that NAC has a lifespan-extending effect in C. elegans (OH et al., 2015). Therefore, it is assumed that different chemical modifications added on cysteine may cause different effects on lifespan. A recent study reports that NAC pre-treatment can cause reduced stress by inducing mitochondrial hormesis (SINGH et al., 2015). Increasing evidence indicates that low dose of ROS can work as a signaling molecule causing an adaptive response, such as mitohormesis, and rather increase lifespan (RISTOW et al., 2014).
Supplementation of S-allyl cysteine...

KIM, J-S.; PARK; S-K.

Figure 4. Effects of SAC on lifespan in *C. elegans*. Age-synchronized worms were counted every day until all worms died to evaluate the effects of SAC on *C. elegans* longevity. There is no significant difference in either mean or maximum lifespan between the wild-type control and 5 μM SAC-treated worms. The values are mean of three independent experiments.

**SAC delays age-related motility decline**

Aging phenotypes in multicellular organisms are tissue-specific. Muscle is one of most vulnerable tissues to age-related accumulation of ROS, due to its high demand on mitochondrial energy metabolism. Based on the *in vivo* antioxidant activity of SAC, we measured the effects of SAC supplementation on motility change with normal aging. At the age of 10 days, 70.6 ± 5.89% (mean of three independent experiments ± SEM) of worms moved freely without any stimuli (level 1) and 29.4 ± 5.89% of worms only moved in response to a mechanical stimulus (level 2) in the wild-type control group. However, SAC treatment significantly altered the ratio of level 1 and level 2; 96.2 ± 1.52% of worms were classified as “level 1” and only 3.8 ± 1.52% of worms were in “level 2” in the SAC-treated worms (*p* = 0.014) (Fig. 5). At the age of 15 days, 23.8 ± 10.99 and 65.7 ±21.77% of worms were categorized as “level 1” in wild-type control and SAC-treated worms, respectively. On day 20, worms in “level 1” increased from only 1.9 ± 1.85% in wild-type control to 10.6 ± 8.53% in SAC-treated worms (Fig. 5). A recent study shows that ROS-scavenging *sesn-1* gene modulates both response to oxidative stress and motility in *C. elegans* (YANG et al., 2013). Deficiency of *sesn-1* results in reduced survival under oxidative-stress conditions and decreased locomotive activity, and over-expression of *sesn-1* confers increased body bending activity. Dietary supplementation of the flavanone derivative, silymarin, leads to increased resistance to oxidative stress and locomotion rate (YANG et al., 2013). Based on our findings, we conclude that nutritional intervention with SAC can promote the health span of *C. elegans* via improvement of body muscle function as well as an increase of resistance to environmental stressors.
Figure 5. SAC treatment delays muscle dysfunction with aging. Time-course change in motility was determined in the wild-type control and SAC-treated worms. Locomotive activity of each worm was categorized as follows: ■ level 1, worms move spontaneously without any mechanical stimulus; ■ level 2, worms move whole body when a stimulus was given; □ level 3, worms move head only in response to a stimulus; □ dead, worms no response to stimulus.

SAC suppresses Aβ toxicity in an Alzheimer’s disease (AD) animal model

AD is an age-related neurodegenerative disease whose pathogenesis is associated with the accumulation of senile plaques composed of Aβ in the brain (KAYED et al., 2003). Transgenic animals expressing human Aβ in muscle show a paralysis phenotype. Induction of the human Aβ gene in C. elegans used as a genetic AD animal model (LINK, 1995). In the present study, we determined the effects of SAC supplementation on paralysis induced by the Aβ transgene. As shown in Fig. 6, the dietary supplementation of SAC significantly suppressed paralysis caused by the expression of Aβ in muscle ($p = 0.006$). At 8 h after Aβ induction, 41.7% of the wild-type worms were paralyzed and only 15.0% of worms treated with SAC were paralyzed. After 11 h of Aβ induction, 83.3% of worms were paralyzed in the wild-type control group, while 48.3% of worms were still not paralyzed in SAC-treated group (Fig. 6). The percent SAC effect on paralysis, calculated using the time taken for 50% of the worms to become paralyzed in each group, was 18.2%. The replicative experiment also showed a significant suppressive effect of SAC on Aβ-induced paralysis ($p = 0.041$, 20.2% effect, data not shown). Using the same C. elegans genetic AD animal model, an insulin/IGF-1-like signal modulates anti-oxidative responses and lifespan and ameliorates toxicity caused by the human Aβ transgene (COHEN et al., 2006). Supplementation of tetracycline significantly retards paralysis by the expression of Aβ (DIOMEDE et al., 2010). These findings suggest that SAC has preventive activity against Aβ toxicity and can be a strong candidate compound for the development of therapeuic agents for AD. Further studies focusing on the identification of other bioactivities of SAC and other cysteine derivatives, and development of novel cysteine derivatives containing increased bioactivity and stability are necessary to apply cysteine derivatives to pharmaceutical and medical use.
Figure 6. Suppression of Aβ-induced toxicity by SAC. The human Aβ transgene was expressed in C. elegans muscle tissues. Induced expression of the Aβ transgene led to paralysis in worms. Supplementation of SAC partially suppresses the onset of Aβ-induced paralysis in the AD animal model. The values are mean of three independent experiments.

ACKNOWLEDGEMENTS

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2015R1D1A1A01057435).

RESUMO: Estudos anteriores mostram que intervenções nutricionais com antioxidantes têm vários efeitos promotores da saúde em vários organismos-modelo. Aqui, examinamos os efeitos da S-álil cisteína sobre a resistência a estressores ambientais e alterações fisiológicas relacionadas com a idade usando C. elegans como um sistema modelo. S-álil cisteína é um aminocácido modificado encontrado em extratos de alho envelhecido e conhecido por ter forte atividade antioxidante. A sobrevida de vermes sob condições de estresse oxidativo aumentou significativamente com a suplementação de S-álil cisteína. Além disso, o pré-tratamento com S-álil cisteína aumentou significativamente a resistência tanto ao estresse térmico como à irradiação ultravioleta. No entanto, o tempo de vida não foi afetado pelo tratamento com S-álil cisteína. Nós também examinamos o efeito da S-álil cisteína na motilidade de C. elegans e descobrimos que a S-álil cisteína pode retardar o declínio relacionado à idade da atividade locomotora do tecido muscular. A S-álil cisteína também suprimiu significativamente a paralisia induzida por amilóide β em animais-modelo da doença de Alzheimer. Tomados em conjunto, o nosso estudo indica que a suplementação dietética de S-álil cisteína pode melhorar a duração da saúde e sugere que S-álil cisteína pode ser usada para desenvolver novos produtos farmacêuticos de promoção da saúde.


REFERENCES


Supplementation of S-allyl cysteine...

KIM, J-S.; PARK; S-K.


Supplementation of S-allyl cysteine...

KIM, J-S.; PARK; S-K.

https://doi.org/10.1073/pnas.232308999

https://doi.org/10.1073/pnas.92.20.9368

https://doi.org/10.1016/S0891-5849(03)00312-5


https://doi.org/10.6061/clinics/2015(05)13


https://doi.org/10.1007/s13273-012-0029-1


Supplementation of S-allyl cysteine...

KIM, J-S.; PARK; S-K.


