Influence of Daidzein and Daidzein Metabolites on Lifespan and Stress Resistance in *Caenorhabditis Elegans*

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Abstract. *Caenorhabditis elegans* (*C. elegans*) was used as an animal model to study the influence of isoflavone daidzein and daidzein metabolites, including dihydrodaidzein (DHD), *O*-desmethylangolensin (*O*-Dma) and equol, on lifespan in *C. elegans* at the concentration of 0.1 mmol/L. The results showed that equol significantly increased the lifespan of *C. elegans*; however, *O*-Dma significantly shortened the lifespan of *C. elegans*. No obvious difference in lifespan was observed when the worms were exposed to daidzein and DHD, respectively. Further study on stress resistance demonstrated that both the thermal and oxidative stress tolerance capacity of *C. elegans* was significantly enhanced after being exposed to equol.

Introduction

Isoflavones, which are mainly composed of daidzein and genistein are functional food factors recognized in soy. Studies have shown that soy isoflavones can exert multiple biological activities, such as antioxidant[1], anti-carcinogenesis[2], a reduced risk of cardiovascular diseases[3], and a potentially protective role for menopausal symptoms and osteoporosis[4]. However, more recently attention has shifted to isoflavone metabolites, which are the products of isoflavone metabolism via specific isoflavone biotransforming bacteria in the gut. Accumulative evidences suggest that daidzein metabolites including dihydrodaidzein (DHD), *O*-desmethylangolensin (*O*-Dma) and equol, are of stronger or wider bioactivities than their precursor isoflavones[5-7]. In our previous study, we have demonstrated that the microbial metabolites of daidzein, including DHD, *O*-Dma and equol showed significantly stronger free-radical scavenging activity than that of daidzein in vitro[8].

Caenorhabditis elegans offers many advantages as a model for biological studies due to its simplicity, transparency, ease of cultivation, and completely sequenced genome[9]. In particular, its short life cycle makes it suitable for aging studies[10]. In the present study, we prepared the microbial metabolites of daidzein and investigated the anti-aging activity of daidzein and daidzein metabolites by using *Caenorhabditis elegans* as a model. In addition, both the thermal tolerance and oxidative stress tolerance were studied. Our results showed for the first time that equol not only significantly increased the lifespan of *C. elegans* but also significantly enhanced the resistance against both thermal and oxidative stress.

Materials and Methods

Chemicals and Reagents

Chemicals daidzein was purchased from Indofine (Somerville, NJ). Daidzein metabolites, including DHD[11]., *O*-Dma[12]. and equol[13]., were prepared by using our previous

microbial biotransformation methods. The enantiomeric excess (% e.e.) of the biosynthesized DHD was zero and that of the biosynthesized *O*-Dma and equol was 88.3% and 100%, respectively. In addition, equol is 100% (−)-*S*-equol. FUDR (5-fluoro-2′-deoxyuridine) was bought from Sigma (98% purity). Juglone (5-hydroxy-1, 4-naphthoquinone, Sigma-Aldrich, 97% purity), a reactive oxygen species-generating compound, was used to induce oxidative stress in worms.

Worms and *Escherichia Coli* OP50

Wild-type *C. elegans* N2 and *Escherichia coli* OP50 used in this study were kindly provided by Professor Chonglin Yang, Chinese Academy of Sciences, China. The worms were maintained and propagated on nematode growth medium (NGM) with standard techniques[14]. Strain *E. coli* OP50 was grown overnight in LB at 37℃ in an incubator followed by being centrifuged (4,000×g) for 10 min at 4℃. The pellet was resuspended in S complete medium to an OD_{600} of approximately 0.90 and stored at 4℃.

Lifespan Analysis

Lifespan assay were performed at 20℃ in a LRH-250A biochemical incubator. Synchronized worms were transferred to 24-well treatment plates when they grew to L4 stage. The worms were then transferred to fresh well every 2 days. Dead worms were scored under NTB-4B stereoscopic microscope every day. A complete absence of swimming movement even after being touched with a small pipette tip was scored as death. Treatment plates were prepared using the reproductive suppressant FUDR (50 μmol/L) and the indicated final concentrations of daidzein and daidzein metabolites was 0.1 mmol/L.

Thermotolerance Assay

Thermotolerance assay was performed with worms on adult day 2. Synchronized L4 worms exposed to 0.1 mmol/L of equol dispersed in live *E. coli* OP50 suspension were maintained at 20℃ and shifted to 35℃ when the thermotolerance assay started. The numbers of surviving and dead worms were scored every 1 h.

Oxidative Stress Assay

Juglone sensitivity was assayed at 20℃ using 1-day-old adults. At that time the worms exposed to 0.1 mmol/L of equol dispersed in live *E. coli* OP50 suspension were transferred onto 24-well plate with 300 μmol/L of juglone.

Statistical Analyses

Statistical analyses were carried out using the Kaplan-Meier method and differences in survival rates were tested using the Log-Rank test with 95% confidence. Analysis of variance in group comparisons was performed by One-way ANOVA and significance of differences between groups were determined by a Tukey's multiple comparison tests. All statistical procedures were carried out in spss18.0 software.

Results

Biosynthesis of Daidzein Metabolites

Daidzein metabolites, including dihydrodaidzein (DHD), O-desmethylangolensin (*O*-dma) and equol, were prepared from the substrate daidzein by using microbial biotransformation method.

Different daidzein metabolites were purified by semi-preparative high-performance liquid chromatography (HPLC). The purity of each biosynthesized DHD, *O*-Dma and equol is more than 99%.

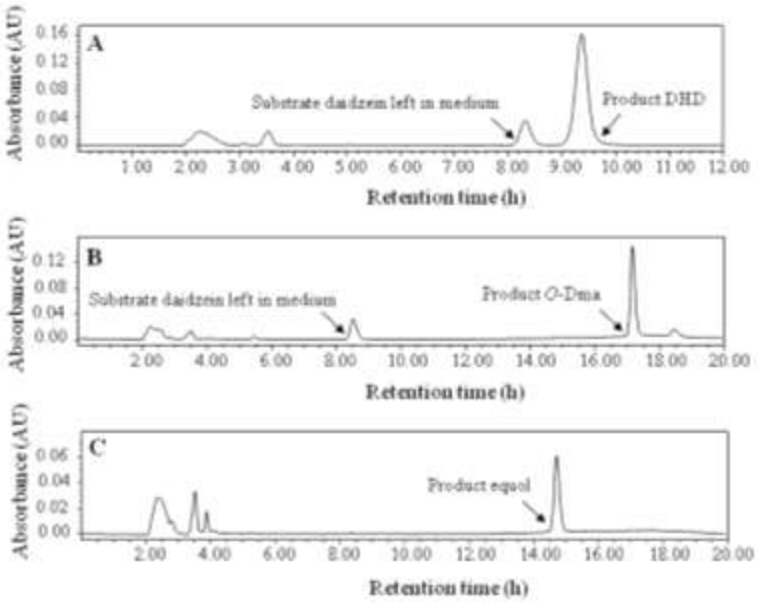

Fig.1 HPLC profiles for biosynthesis of daidzein metabolites

Influence of Daidzein and Daidzein Metabolites on the Lifespan of C. elegans N2

To determine whether daidzein and daidzein metabolites could affect the worm's lifespan, we presented daidzein and daidzein metabolites, including DHD, *O*-Dma and equol, to the worms (Fig.2). The mean lifespan (in mean number of days ± standard error) of the control worms fed the standard laboratory food OP50 with 0.1 mmol/L of daidzein was 11.900 ± 1.154 d; that of the worms exposed to 0.1 mmol/L of DHD, *O*-Dma and equol were 11.100 ± 1.350 d, 9.467 ± 0.859 d and 15.300 ± 1.079 d, respectively. Statistical analysis indicated that the mean lifespan of the worms exposed to 0.1 mmol/L of equol ($P < 0.05$) was significantly longer than those of the control worms exposed to 0.1 mmol/L of daidzein. However, on the contrary, the mean lifespan of the worms exposed to 0.1 mmol/L of *O*-Dma ($P < 0.05$) was significantly shortened. No obvious difference in lifespan was observed when the worms were exposed to the same concentration of DHD. In addition, we did not observe any change in body size when the worms exposed to daidzein and different daidzein metabolites in comparison with the control worms fed with OP50.

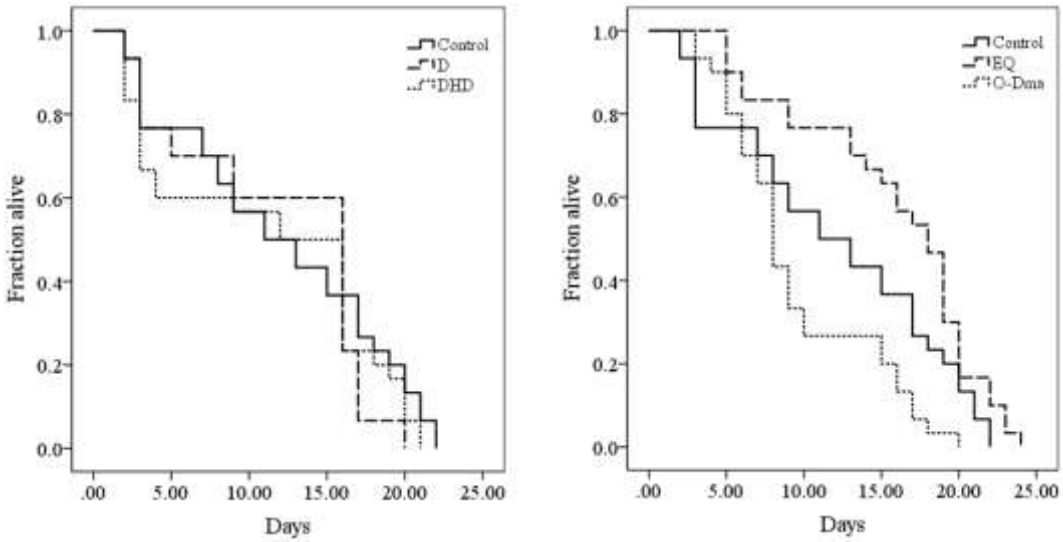

Fig.2 Effect of daidzein and daidzein metabolites on the lifespan of *C. elegans*

Influence of Equol on Thermal Stress Resistance in C. elegans N2

In order to investigate whether the increased longevity was associated with the improved survival capability under stress condition, we detected the thermal stress resistance of the worms exposed to 0.1 mmol/L (Fig.3). The results showed that the mean survival time for the control and treated worms at 35℃ were 13.467 ± 0.782 h and 15.933 ± 0.835 h ($P < 0.05$) respectively.

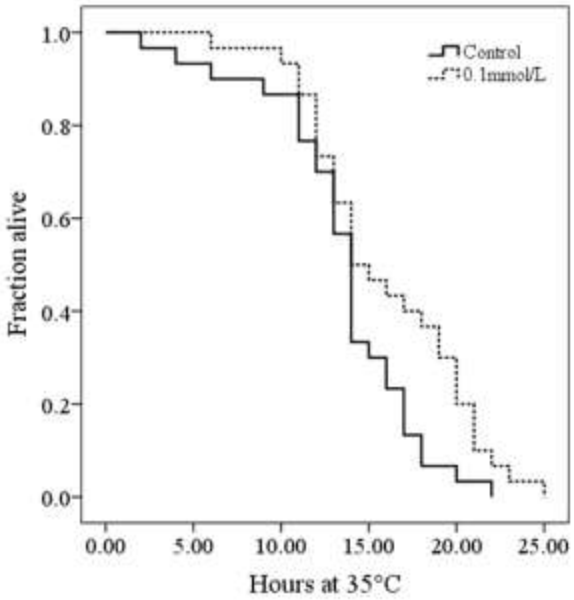

Fig.3 Effect of equol on resistance against thermal of *C. elegans*

Influence of Equol on Oxidative Stress Resistance in C. elegans N2

To evaluate the potential effect of equol on wild-type *C. elegans* N2 under oxidative stress, the worms were exposed to 300 μmol/L of juglone after being treated with 0.1 mmol/L of equol for 1 days (Fig.4). When the worms exposed to 0 mmol/L of equol (the control) and 0.1 mmol/L of equol (the treatment) were continuously exposed to juglone for 10 h, the average

survival rate were 26.7% and 63.3%, respectively. Statistical analysis indicated that equol treatment significantly improved the mean survival of *C. elegans* under juglone-induced oxidative stress ($P < 0.01$).

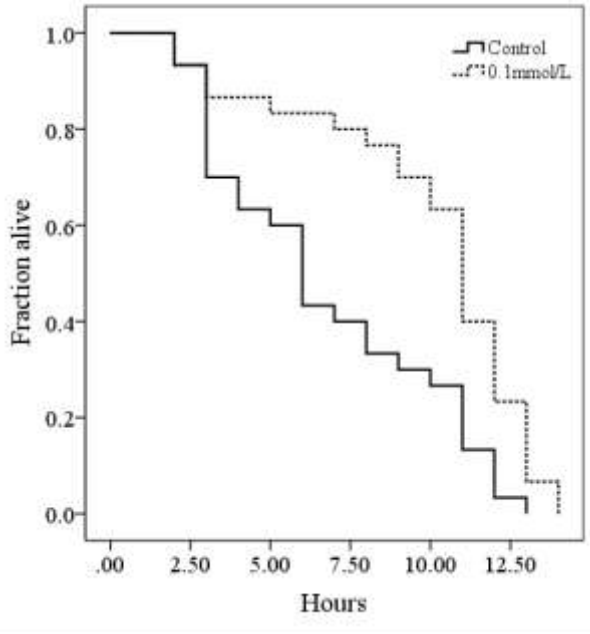

Fig.4 Effect of equol on resistance against oxidative stress of *C. elegans* caused by juglone

Conclusions

Our study demonstrated that equol not only significantly increased the lifespan of *C. elegans* but also significantly enhanced the resistance against both thermal and oxidative stress at the concentration of 0.1 mmol/L. Neither daidzein nor DHD influenced the lifespan of *C. elegans*; however, *O*-Dma significantly shortened the lifespan of *C. elegans* at the concentration of 0.1 mmol/L.

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References

[1] C.E. Rüfer, S.E. Kulling, Antioxidant activity of isoflavones and their major metabolites using different in vitro assays, J. Agri. Food Chem. 54 (2006) 2926-2931.

[2] S. Widyarini, A.J. Husband, V.E. Reeve, Protective effect of the isoflavonoid equol against hairless mouse skin carcinogenesis induced by UV radiation alone or with a chemical cocarcinogen, Photochem. Photobiol. 81 (2005) 32-37.

[3] S. Kreijkamp-Kaspers, L. Kok, M.L. Bots, G.D.E. robbee, J.W. Lampe, Y.T. van der Schouw, Randomized controlled trial of the effects of soy protein containing isoflavones on vascular function in postmenopausal women, Am. J. Clin. Nutr. 81 (2005) 189-195.

[4] S. Levis, N. Strickman-Stein, P. Ganjei-Azar, P. Xu, D.R. Doerge, J. Krischer, Soy isoflavones in the prevention of menopausal bone loss and menopausal symptoms, Arch. Intern. Med. 171 (2011) 1363-1369.

[5] T.E. Hedlund, P.D. Maroni, P.G. Ferucci, R. Dayton, S. Barnes, K. Jones, R. Moore, L.G. Ogden, K. Wähälä, H.M. Sackett, K.J. Gray, Long-term dietary habits affect soy isoflavone metabolism and accumulation in prostatic fluid in caucasian men, J. Nutr. 135 (2005) 1400-1406.

[6] M. Uehara, Isoflavone metabolism and bone-sparing effects of daidzein-metabolites, J. Clin. Biochem. Nutr. 52(2013) 193-201.

[7] T.E. Hedlund, W.U. Johannes, G.J. Miller, Soy isoflavonoid equol modulates the growth of benign and malignant prostatic epithelial cells in vitro, Prostate. 54 (2003) 68-78.

[8] X.L. Liang, X.L. Wang, Z. Li, Q.H. Hao, S.Y. Wang, Improved in vitro assays of superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of isoflavones and isoflavone metabolites, J. Agric. Food Chem., 58 (2010) 11548-11552.

[9] D.L. Riddle, T. Blumenthal, B.J. Meyer, J.R. Priess (ed.), C. elegans II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1997.

[10] E. Kimberley, J.C. James, H. Cheng, H. Stacie, K. Kerry, Valproic acid extends Caenorhabditis elegans lifespan, Aging Cell. 7 (2008) 305-317.

[11] H. Zhao, X.L. Wang, H.L. Zhang, C.D. Li, S.Y. Wang. Production of dihydrodaidzein and dihydrogenistein by a novel oxygen-tolerant bovine rumen bacterium in the presence of atmospheric oxygen, Appl. Microbiol. Biotechnol., 92 (2011) 803-813.

[12] X.L. Wang, J.Z. Shao, S.Y. Wang, F. Yu, Q. Zhang, C.D. Li, Chinese patent ZL 200810147314.9 (2011)

[13] M. Li, C.H. Zhang, X.L. Wang, B.H. Chen, Q.H. Hao, S.Y. Wang, Enhanced biosynthesis of O-desmethylangolensin from daidzein by a novel oxygen-tolerant cock intestinal bacterium in the presence of atmospheric oxygen, J. Appl. Microbiol. 118 (2015) 619-628.

[14] S. Brenner, The genetics of Caenorhabditis elegans, Genetics. 77 (1974) 71-94.