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Sesamin and sesamolin reduce amyloid-β toxicity in a transgenic *Caenorhabditis elegans*

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ABSTRACT

Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by β-amyloid (Aβ) plaques in the brain. At the present, there is no approved drug with a proven disease-modifying effect. Sesame seed (*Sesame indicum*) has long been known as a healthy food in Southeast Asian countries. Sesame lignans obtained from sesame seed possess antioxidant property that exhibit a variety of beneficial effects in various models. The objective of this study was to investigate the protective effects of sesame lignans including sesamin, sesamolin, and sesamol against Aβ toxicity in *Caenorhabditis elegans* (*C. elegans*) model of Aβ toxicity and to address whether these sesame lignans have a positive effect on lifespan extension. A transgenic *C. elegans* expressing human Aβ was used to investigate protective effects of sesame lignans against Aβ toxicity. Sesamin and sesamolin significantly alleviated Aβ-induced paralysis. The real-time PCR revealed that both sesamin and sesamolin did not affect the expression of Aβ transgene. However, we found that only sesamin inhibited Aβ oligomerization. These findings demonstrated that, among three sesame lignans tested, sesamin protected against Aβ toxicity by reducing toxic Aβ oligomers. Sesamin and sesamolin also significantly improved Aβ-induced defect in chemotaxis behavior and reversed the defect to normal. Moreover, sesamin prolonged median and mean lifespan of the wild type worm. On the other hand, sesamolin and sesamol failed to extend lifespan. These results offer valuable evidence for the future use of sesamin in the development of agents for the treatment of AD. It is also worth investigating the structure-activity relationship of lignan-related structures and their anti-Aβ toxicity activities in the future.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized clinically by the impairment in cognitive function and pathologically by the deposition of β-amyloid (Aβ) plaques as well as neurofibrillary tangles (NFTs) in the brain [1,2]. AD is the most common cause of dementia. As the aging population increases, it has been predicted that the number of people with dementia will rise from 24.3 million people in 2001 to 42.3 million in 2020 and 81.1 million by 2040 [3]. As such, without the effective means to prevent or cure the disease, the number of AD patients will be dramatically increased. Therefore, the mean to prevent or reduce the rate of this disease is a high priority for medical research.

Modeling Aβ toxicity in *Caenorhabditis elegans* (*C. elegans*) was initiated around two decades ago [4–7]. The model is based on the amyloid cascade hypothesis proposing that increased production of Aβ is central to AD pathology. In a transgenic *C. elegans* model, human Aβ fragment has been expressed intracellularly in the body wall muscle. This model has a clear phenotype which is a progressive paralysis, typically beginning in young adulthood [4]. This paralysis is attributed to the accumulation of intracellular Aβ deposit in the muscle cells. Although Aβ expression is limited to the muscle cells, this transgenic *C. elegans* establishes a relationship between intracellular Aβ accumulation and Aβ toxicity. The transgenic *C. elegans* expressing Aβ in neuron has also been developed by Link [4]. The behavioral phenotype of this transgenic worm has been identified. This worm demonstrated deficits in odorant preference associative learning behavior and hypersensitivity to serotonin [8,9]. *C. elegans* model has also helped to identify the

Abbreviations: AD, Alzheimer's disease; Aβ, β-amyloid; *C. elegans*, *Caenorhabditis elegans*; DMSO, dimethyl sulfoxide; PT_{50}, median paralysis time

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connection between proteotoxicity in neurodegenerative disease and lifespan extension pathway [10,11]. A positive correlation between lifespan extension and protection against Aβ has also been established. Aβ toxicity was reduced in *C. elegans* when aging was slowed by decreased insulin/insulin growth factor-1-like signaling (IIS) [10].

Sesame seed has been known as a healthy food in Southeast Asian countries [12]. Sesame seeds and its oil contain important bioactive compounds, a group of lignans, which plays an important role in health-promoting effect. Lignans are compounds consisting of dimers of phenyl propane unit. They are found in a variety of plants. Sesame seed is the main source of antioxidant lignans such as sesamin, sesamolin, and sesamol. These lignans exhibited beneficial effects in various models. Sesamin and sesamolin inhibited endogenous lipid peroxidation as well as oxidative DNA damage in rat liver and kidney [13]. They also possessed scavenging property of peroxy radical *in vitro* [14,15], and showed inhibitory effects on susceptibility to oxidative stress in hypercholesterolemia rabbit, and on hypoxia-induced pheochromocytoma and neuronal cell death [16,17]. Sesamin and sesamolin possessed neuroprotective effects against hypoxia or brain damage [18]. Sesamol was a powerful antioxidant that inhibited ultra violet and Fe^{3+}/ascorbate-induced lipid peroxidation in rat brain [19]. Sesamol also possessed neuroprotective, hepatoprotective, anti-inflammatory, chemo-preventive and anti-aging properties [19–23].

In the present study, we investigated the protective effect of sesame lignans, including sesamin, sesamolin, and sesamol, against Aβ toxicity using *C. elegans* model of Aβ toxicity and addressed whether these sesame lignans have a positive effect on lifespan extension.

2. Materials and methods

2.1. Plant extract and treatment

Ginkgo biloba extract (EGb 761) (Batch number: PSC0148/Ch.454) was generously provided by Dr. Willmar Schwabe Pharmaceuticals. Sesamin and sesamol were purchased from Spectrum Chemical (Gardena, CA, USA). Sesamolin was from Cayman (Ann Arbor, MI, USA). Stock solutions of sesame lignans and EGb 761 were made either with dimethyl sulfoxide (DMSO) or distilled water. Sesame lignans and EGb 761 were added directly to the OP50 food source to a desired final concentration. The treatment was given to the worms from the egg stage onward. The structures of sesame lignans are shown in Fig. 1.

2.2. Caenorhabditis elegans strains and maintenance

C. elegans strains N2, CL4176, CL802, CL2355, and CL2122 were obtained from the Caenorhabditis Genetic Center (CGC, University of Minnesota). The worms were maintained at 20 °C (for N2) and 16 °C (for CL4176, CL802, CL2355, and CL2122) on a nematode growth media (NGM) plate with *E. coli* strain OP50 as a food source. Age-synchronized worms were prepared by transferring the reproductive worms (3 days of age) to the fresh NGM plates and permitted to lay eggs for 4–6 h. The eggs were then allowed to hatch and used in the experiments.

2.3. Paralysis assay

Transgenic *C. elegans* strain CL4176 and CL802 was age-synchronized onto the 60 × 10 mm culture plates spotted with OP50 containing either a vehicle or sesame lignans. For *C. elegans* strain CL4176, Aβ transgene expression in muscle cells was induced by up-shifting the temperature from 16 °C to 25 °C at the 36th h after egg laying and maintained until the end of the paralysis assay. After temperature upshift for 24 h, the number of paralyzed worms was scored under the microscope at one hour intervals until the last worm became paralyzed. To identify the paralysis, each worm was gently touched with a platinum loop (VWR, Bridgeport, NJ). The worm was considered paralyzed if it did not move or moved head only after touching. Paralysis time course was plotted.

2.4. RNA extraction and real-time PCR

Transgenic *C. elegans* strain CL4176 worms were incubated at 16 °C until they reached L3 stage, then up-shifted to 25 °C, and harvested at 33 h after being up-shifted. Worms were washed by PBS and collected. Worm pellets were snap frozen in liquid nitrogen and thawed two times. RNA extraction was performed by following Trizol/RNeasy hybrid RNA extraction protocol using the RNeasy® Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized using qScript™ XLT cDNA SuperMix (Quanta Biosciences) by following the manufacturer's instruction. Real-time PCR was performed in a PCRmax ECO 48 Real-time qPCR system (United Kingdom) using PerfeCTa SYBR Green FastMix (Quanta Biosciences). The primers used in the quantitative PCR were as followed: *actin-1*(F) 5′−CCAGGAATTGCTGATCGTATGCAGAA-3′ and (R) 5′-TGGAGAGGGAAGCGAGGATAGA-3′; Aβ transgene (F) 5′−CCG ACATGACTCAGGATATGAAGT-3′ and (R) 5′-CACCATGAGTCCAATGA TTGCA-3′. Cycling conditions were 95 °C × 3 min, followed by 40 cycles of 95 °C × 5 s + 55 °C × 15 s. The relative quantification of mRNA was analyzed using the $2^{-\triangle\triangle Ct}$ method.

2.5. Western blotting

The Aβ species in the transgenic *C. elegans* strain CL4176 was identified by immunoblotting using a Tris-Tricine gel and the standard Western blotting protocol. *C. elegans* strain CL802 was used as a negative control of the expression of Aβ. After the experimental treatments, the worms were collected by washing three times with 1 × PBS and quickly frozen in liquid nitrogen. The worms were sonicated in lysis buffer (20 mM Tris-HCL pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β–glycerophosphate, 1 mM Na3VO4, 1 μg/ml leupeptin)

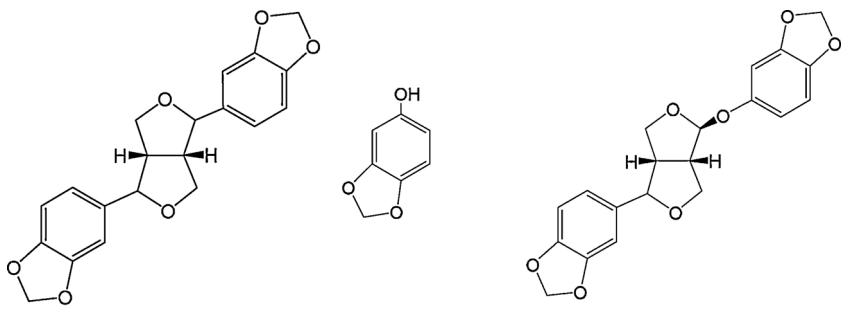

Fig. 1. Structures of sesame lignans.