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Baicalein modulates stress-resistance and life span in *C. elegans* via SKN-1 but not DAF-16

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ABSTRACT

The flavonoid baicalein has been demonstrated to be an activator of the transcription factor Nrf2 in mammalian cell lines. We show that it further modulates the Nrf2 homolog SKN-1 in *Caenorhabditis elegans* and by this pathway mediates beneficial effects in the nematode: baicalein enhances the resistance of *C. elegans* against lethal thermal and sodium arsenite stress and dose-dependently prolongs the life span of the nematode. Using RNA interference against SKN-1 we were able to show that the induction of longevity and the enhanced stress-resistance were dependent on this transcription factor. DAF-16 (homolog to mammalian FOXO) is another pivotal aging-related transcription factor in the nematode. We demonstrate that DAF-16 does not participate in the beneficial effects of baicalein: since baicalein causes no increase in the nuclear translocation of DAF-16 (DAF-16::GFP expressing strain, incubation time: 1 h) and it still induces longevity even in a DAF-16 loss-of-function strain, we conclude, that baicalein increases stress-resistance and life span in *C. elegans* via SKN-1 but not DAF-16.

1. Introduction

Baicalein (Fig. 1) is a flavonoid derived from Baical skullcap (*Scutellaria baicalensis*), a plant widely used in traditional folk medicines for the treatment of e.g. inflammation, cardiovascular illnesses and gastrointestinal infections [1]. In addition to these traditional dosage forms, there are also dietary supplements available containing either extracts of *Scutellaria baicalensis* or baicalein as pure compound. Due to extraordinate structural element of three proximate hydroxygroups in ring A, baicalein has potent radical-scavenging effects [2] and has previously been shown to mediate antioxidative effects in mammalian cells by activation of the Nrf2 signaling pathway [3–8]. Using *Caenorhabditis elegans* it has been shown that baicalein reduces ROS accumulation and prolongs the life span of this model organism: baicalein extends the mean, median and maximum lifespans by 45, 57 and 24%, respectively [3]. However, the molecular mechanism for the beneficial effects of baicalein in the nematode is not clear.

C. elegans is a well-established model in genetics [9] but is currently also used to analyze biological effects of natural compounds in a complete, simple structured organism [10–12]. *C. elegans* possesses a homology to 60–80% of the human genome [13], especially a high number of signaling pathways are conserved in this organism [14]. This predestinates the nematode as a useful and applicable model to study molecular mechanisms of natural compounds in a simple organism. The *C. elegans* transcription factor SKN-1 is the functional homolog of the mammalian Nrf2 [15,16]. Comparable to Nrf2, SKN-1 is activated by e.g. oxidative stress or xenobiotics and then translocates into the nucleus where it binds to the antioxidant responsive element (ARE) in the promotor regions of various antioxidative or protective genes [17]. A high number of Nrf2-regulated genes possess homologs in *C. elegans* which are regulated by SKN-1 [18]. Activation of this transcription factor is associated with a prolongation of life span [19] and increase in stress resistance of the nematode [20]. It has been discussed, that in analogy to the effects of baicalein on Nrf2 signaling in mammalian cells and due to the observed modulation of the SKN-1-localization by baicalein, this flavonoid might induce life prolongation in *C. elegans* via the transcription factor SKN-1[3]. Now we have used RNA interference and transgenic strains to clarify this hypothesis. Additionally, we further evaluated the role of DAF-16, another important life span modulating transcription factor in this process.

Abbreviations: ARE, antioxidant response element; BSA, bovine serum albumin; DCF, 2′, 7′-dichlorofluorescein; DMSO, dimethyl sulfoxide; FUDR, 5-fluorodeoxyuridine; IPTG, Isopropyl β-D-1-thiogalactopyranoside; NGM, nematode growth medium; ROS, reactive oxygen species; TCM, Traditional Chinese Medicine.

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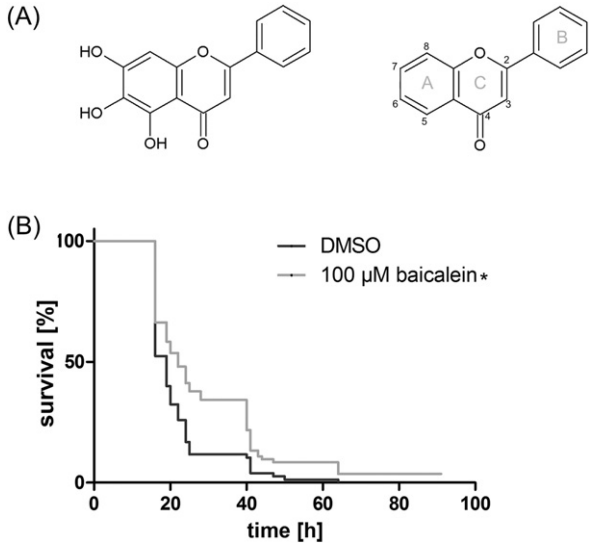

Fig. 1. Baicalein enhances the resistance of *C. elegans* to sodium arsenite stress: (A) structure of the flavonoid baicalein, right: general flavonoid structure. (B) Sodium arsenite assay: 3 days old synchronized nematodes were treated with 100 μM baicalein or 0.1% DMSO for 3 days and were then exposed to 5 mM sodium arsenite. Their survival was monitored by touch-provoked movement. Kaplan-Meier statistics were performed with a total of 84 or 89 nematodes per treatment group in three independent experiments.

2. Material and methods

2.1. Materials

2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and baicalein (≥98%) were obtained from Sigma (Deisenhofen, Germany). All other chemicals were used in the highest purity available and obtained from Sigma (Deisenhofen, Germany) of Roth (Karlsruhe, Germany). The stock solution of baicalein (100 mM) was prepared in DMSO; 0.1% of DMSO was used as vehicle control.

2.2. Maintenance of C. elegans

N2 wild type *C. elegans*, transgenic TJ356 [*zIs356 IV* (*pdaf-16-daf-16::gfp; rol6*)] and CF1038 [*daf-16*(*mu86*) *I.*] *C. elegans*, as well as the OP50 and streptomycin resistant OP50-1 *Escherichia coli* strains were obtained from the *Caenorhabditis* Genetics Centre (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). RNAi feeding bacteria HT115 were obtained from SourceBiosystems (Nottingham, UK). The nematodes were maintained at 20 °C on nematode growth medium (NGM) plates seeded with OP50 as a food source as described by Lewis and Fleming [21]. Age-synchronized cultures were achieved by placing gravid adults on fresh NGM plates seeded with OP50 and allowing them to lay eggs for a time span of max. 3 h. After this the adults were removed and the eggs were allowed to hatch and develop to larval stadium L4 or young adult before incubation for the experiments. For the RNAi experiments, NGM plates supplemented with 1 mM IPTG, 0.1 mg/ml ampicillin and 12.5 μg/ml tetracyclin were used and were seeded with HT115. Incubations were performed at 20 °C in liquid NGM containing 1% BSA, 50 μg/ml streptomycin and 1 × 10 [9] OP50-1/ml as food source. For the RNAi feeding the liquid NGM was supplemented with 1% BSA, 1 mM IPTG, 0.1 mg/ml ampicillin, 12.5 μg/ml tetracyclin and 1 × 10 [9] HT115/ml as food source.

2.3. Assessment of stress resistance

(A) Sytox®Green assay: age-synchronized N2 (normal or RNAi conditions) were incubated with 100 μM baicalein or vehicle control for 2.5 days with daily transfer to new medium. After washing in PBST (PBS with 0.1% Tween 20) the nematodes were individually transferred into the wells of a black 384-well plate with a clear bottom. A final concentration of 1 μM Sytox®Green nucleic acid stain (molecular probes) was added, the plate sealed against evaporation and thermal stress (37 °C) was applied. The increase in fluorescence was measured every 15 min for a time span of 12 h (excitation wavelength: 485 nm, emission wavelength: 535 nm) using a Wallace Victor^2 1420 multilabel counter (Perkin Elmer). Virtual death points were calculated as the time points at which the fluorescence of a nematode exceeded the mean increase of the first 3 time points by a factor of 3.

(B) Sodium arsenite-induced stress: age-synchronized nematodes (N2) were incubated with 100 μM baicalein or vehicle control for 2.5 days in liquid NGM supplemented additionally with 120 μM FUDR. The nematodes were shifted to fresh medium on the morning of day 2. After incubation the worms were transferred to M9 buffer containing 5 mM sodium arsenite, 120 μM FUDR and 1 × 10 [9] OP50-1/ml. Survival of the worms was monitored regularly during day time using touch-provoked movement. The nematodes were examined by touching first posterior and then anterior. Non-responding *C. elegans* were finally cut in two. Nematodes showing extruded internal organs were censored at the time point of the event.

2.4. DAF-16::GFP translocation

Age-synchronized transgenic TJ356 *C. elegans* were incubated for 1 h with 100 μM baicalein or vehicle control, respectively. The nematodes were then mounted onto microscope slides, anesthetized with 10 mM NaN_3 and were then analyzed concerning visibility of GFP-fluorescence in the nuclei.

2.5. Pharynx pumping assay

Age-synchronized nematodes (N2) were incubated with 100 μM baicalein or vehicle control, respectively, were kept at 25 °C and were transferred to fresh medium every day. Pharynx pumping activity was monitored on day 5, 10 and 15 for 30 s.

2.6. Life span analysis

Age-synchronized N2 nematodes (normal or RNAi conditions) or TJ356 *C. elegans* (RNAi conditions) were incubated with various concentrations of baicalein or vehicle control (the amount of DMSO was kept constant at 0.1%), and were kept at 25 °C. Survival was examined daily

Table 1
Baicalein prolongs the life span of *C. elegans*.

	Median life span (days)		Number of nematodes	Number of censored nematodes	*p* value vs. control
DMSO	23	100.0%	114	21	–
25 μM baicalein	25	108.69%	115	20	0.214
50 μM baicalein	27	117.39%	114	22	0.001
100 μM baicalein	36	156.52%	114	19	0.001