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Molecular Effects of Baicalein in Hct116 Cells and *Caenorhabditis elegans*: Activation of the Nrf2 Signaling Pathway and Prolongation of Lifespan

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ABSTRACT: Baicalein is a major compound of extracts derived from *Scutellaria baicalensis* Lamiaceae, which are used as food supplements. Baicalein possesses a high radical scavenging activity and decreases intracellular reactive oxygen species in Hct116 human colon carcinoma cells and in *Caenorhabditis elegans*. It activates Nrf2, a key transcription factor that binds to the antioxidant responsive element (ARE): Baicalein causes a nuclear accumulation of Nrf2, increases ARE-dependent luciferase activity, and enhances the expression of heme oxygenase-1 in Hct116 cells. Additionally, accumulation of the Nrf2 homologue SKN-1 in nuclei of intestinal cells of *C. elegans* was observed. Lifespan analysis revealed that baicalein extends the mean, median, and maximum lifespans of the nematode by 45, 57 and 24%, respectively. Because SKN-1 activation is associated with prolongation of lifespan, the results suggest that baicalein increases the lifespan of *C. elegans* by activation of the Nrf2/SKN-1 signaling pathway.

KEYWORDS: *baicalein, Caenorhabditis elegans, flavonoid, Nrf2, SKN-1, Hct116 cells*

INTRODUCTION

Baicalein (5,6,7-trihydroxyflavone; Figure 1) is a flavonoid derived from *Scutellaria baicalensis* (skullcap). Decoctions and

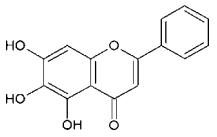

Figure 1. Structure of the flavonoid baicalein.

tinctures prepared from the root are known as "Huangqin"[1] and are widely used in traditional Chinese medicine for the treatment of fever,[2] viral and bacterial infections,[3,4] and inflammation[5] as well as cancer.[6] In addition to these traditional dosage forms (Huangqin or as a tea), there are also dietary supplements available. If taken as a supplement, baicalein intake can be estimated as up to 5 mg/day.[4] A major compound detected in most *S. baicalensis* products is baicalein-7-*O*-glucoronide (baicalin).[4] This compound can be cleaved by β-glucuronidases of the intestinal microflora, which results in a higher baicalein exposure in the colon.[7] Baicalein has been reported to possess an antioxidant activity via scavenging of free radicals.[8,9] In addition to this direct antioxidant activity, flavonoids may act as antioxidants indirectly: Indirect antioxidative effects can be mediated (i) by chelation of redoxactive metal ions or (ii) modulation of redox active signaling pathways and consistently increased expression of antioxidative enzymes. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a redox-active transcription factor that binds to the antioxidant responsive element (ARE), a distinct DNA motif in the promoter region of various antioxidant and phase II genes, for example, heme oxygenase-1 (HO-1) and glutathione *S*-transferase (GST). In physiological conditions this transcription factor is located in the cytosol, where it is associated with the inhibitor protein Kelch-like ECH-associated protein 1 (Keap1). This inhibitor protein is an adaptor for the Cul3 ubiquitin E3 ligase system and therefore modulates the kinetic of proteasomal degradation.[10] Keap1 also acts as a sensor for the cellular redox status: Oxidation of distinct cysteine residues results in stabilization of Nrf2[11] and accumulation of the transcription factor in the nucleus, where it dimerizes with small Maf proteins.[10] The complex of Nrf2 and Maf proteins mediates transcriptional activation by binding to AREs in the promoter regions of various genes encoding antioxidant[12] and phase II drug metabolizing enzymes[13] as well as transport proteins.[10] Besides oxidation of Keap1, the Nrf2/ARE pathway is modulated by various protein kinases, for example, ERK, JNK, and p38.[11] Due to the induction of antioxidant enzymes, the Nrf2 signaling pathway is a key cellular defense mechanism against oxidative stress, and therefore activation of this pathway is associated with beneficial effects. Activation of the Nrf2 signaling pathway builds up a more prolonged defense system compared to uptake of compounds that act only by scavenging radicals.[14] For example, HO-1 is regulated via Nrf2 signaling. This enzyme plays a crucial role in degradation of the heme molecule to CO, iron,

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and biliverdin, thus maintaining cellular homeostasis. A modulation of the Nrf2/ARE signaling pathway by specific flavonoids has been reported before;[14] an activation of this pathway by baicalein has previously been shown in V79 Chinese hamster lung fibroblasts[15] and PC12 rat pheochromocytoma cells.[16] We investigated the impact of baicalein on the Nrf2/ARE signaling pathway using Hct116 human colon carcinoma cells as a model system for a primary target organ of this flavonoid. Additionally, we investigated the effects of baicalein on the Nrf2 homologue signaling pathway in a living organism, the nematode *Caenorhabditis elegans*. Compared to results from cell culture experiments, results from this model organism may be more relevant for an estimation of the biological effects of this flavonoid in humans.

MATERIALS AND METHODS

Materials. 2,7-Dichlorodihydrofluorescein diacetate (H_2DCF-DA) and baicalein were obtained from Sigma (Deisenhofen, Germany). Hct116 human colon carcinoma cells (obtained from the DSMZ, Braunschweig, Germany) were cultured at 37 °C in a humidified atmosphere (5% CO_2). DMEM (high glucose) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin was used as cell culture medium. The ARE GST-Ya luciferase reporter gene vector kindly provided by Dr. Ming Zhu (UC Davis Cancer Center, Davis, CA, USA) was constructed as described elsewhere.[17] The vector for the Nrf2::GFP fusion protein was constructed by insertion of the complete Nrf2 CDS between the *Bam*HI site and the *Xho*I site of the C1-pEGFP vector. The Nrf2 fragment was generated using the following primers: 5′Nrf2 (5′-ctcgagtaatgatggacttggagctgccg-3′), 3′Nrf2 (5′-ctagtttttcttaacatctgggga-tcc-3′) introducing a *Bam*HI site and a *Xho*I site. The fragment was subcloned in pGEMTEasy (Promega, Germany), extracted by using *Bam*HI and *Xho*I to improve the added restriction sites. N2 wild type *C. elegans*, transgenic *C. elegans* LD001 (Pskn-1::SKN-1::gfp; rol6), OP50, and streptomycin-resistant OP50-1 *Escherichia coli* strains were obtained from the *Caenorhabditis* Genetics Centre (University of Minnesota). Nematodes were maintained at 20 °C on nematode growth medium (NGM) plates with OP50 as a food source as described by Lewis and Fleming.[18]

Methods. *TEAC Assay.* The ABTS radical solution was prepared using equal volumes of an ABTS solution (14 mM) and a 4.9 mM APS solution, which was diluted with ethanol (80%) to get an absorption of 1.4 (734 nm). Dilutions of Trolox and baicalein were prepared in 80% ethanol. One volume of sample solution and one volume of the ABTS solution were mixed, and the decrease of absorbance was monitored spectrophotometrically 2 min after mixing.

*DCF Assay. (A) DCF Assay in Hct116 Cells.*Cells were seeded into 6-well plates (5×10^5 cells/well) and allowed to attach for 24 h. Cells were treated with 25 μM baicalein or DMSO as vehicle control for 4 h, washed with medium, and then incubated with H_2DCF-DA (10 μM) for 15 min. After a washing step, cells were incubated with 500 μM H_2O_2 for 1 h and then harvested. Flow cytometric analysis was performed using an Accuri C6 flow cytometer (Accuri Cytometers, St. Ives, Cambs, UK): excitation, 488 nm; emission, 530 ± 15 nm. *(B) DCF Assay in C. elegans.* Synchronization of the culture was achieved by placing gravid adults on NGM plates and allowing them to lay eggs for up to 3 h. After this time, the adults were removed and the eggs were allowed to hatch and develop to larval stage L4 or young adult before incubation with 100 μM baicalein or vehicle control, respectively. Incubation was carried out at 20 °C in liquid NGM containing 1% BSA, 50 μg/mL streptomycin, and 1×10^9 OP50-1/mL as a food source. After 2 days of treatment (incubation medium was changed daily), nematodes were individually transferred into the wells of a 384-well plate containing M9 buffer. H_2DCF-DA was added to a final concentration of 50 μM, and the plate was sealed against evaporation. Thermal stress (37 °C) was applied, and an increase of DCF fluorescence was measured using a Wallace Victor^2 1420 multilabel counter (excitation, 485 nm; emission, 535 nm) immediately after the onset of the stress conditions.

Intracellular Localization of Nrf2. (A) Fluorescence Microscopy. Transient transfection with Nrf2::GFP vector was performed using TurboFect (Fermentas) according to the manufacturer's protocol. Briefly, Hct116 cells were seeded into 12-well plates at a density of 1.5×10^5 cells/well and allowed to attach for 24 h. Complexes were formed at a DNA to reagent ratio of 0.5. Transfection was performed for 24 h, and then cells were allowed to recover for 24 h. Cells were incubated with 40 μM baicalein or the equivalent volume of DMSO for 4 h before fixation in ice-cold methanol. Nuclei were stained with Hoechst 33342, and the slides were analyzed using a fluorescence microscope (Hoechst filter excitation at 365 nm and emission at 420; GFP filter excitation at 450−490 nm and emission at 515 nm). *(B) Western Blot Analysis.* Hct116 cells were seeded into 6-well plates (7.5×10^5 cells/well) and were allowed to attach for 24 h. Cells were treated with different concentrations of baicalein for 4 h, and then proteins were isolated. (i) Total protein: cells were washed with PBS and suspended in ice-cold RIPA buffer. After two freeze−thaw cycles, lysates were centrifuged and the supernatant containing total proteins was collected. (ii) Nuclear and cytosolic fraction: cells were washed with PBS and lysed for 15 min in ice-cold buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, proteinase inhibitor cocktail, and 0.01% okadaic acid). Then Nonidet-P40 was added, and the suspension was vortexed for 1 min. After centrifugation, the supernatant containing the cytosolic fraction was collected. The pellet was rinsed in ice-cold PBS and then stirred in buffer B (20 mM HEPES, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF) at 4 °C for 25 min before centrifugation. The resulting supernatant contained the nuclear fraction.

Protein content was measured using a Bio-Rad DC protein assay according to the instructions of the manufacturer. Proteins were separated by SDS-PAGE and were then transferred to a PVDF Western blot membrane (Roche, Mannheim, Germany; 40 μg total protein (70 μg for HO-1 detection), 30 μg nuclear protein, or 60 μg of cytosolic protein). Membranes were blocked (5% BSA in TBS supplemented with 0.1% Tween 20) for 1 h at room temperature and then incubated with anti-Nrf2 (1:5000, Epitomics, Burlingame, CA, USA) or anti-HO-1 (1:1000, Epitomics) antibodies, respectively. Goat anti-rabbit (1:3000, SouthernBiotech, Birmingham. AL, USA) was used as secondary antibody. Signals were visualized using a BM Chemiluminescence Western Blotting Kit (Roche) and detected using a Fusion FX7 imaging system (Peqlab, Erlangen, Germany). Densitometric analysis was performed using Peqlab Bio1D software.

Reporter Gene Assay. Transient transfection was performed with a batch protocol using JetPei transfection reagent (Polyplus Transfection) according to the manufacturer's protocol. In brief, complex formation was achieved using 2 μL of transfection reagent per microgram of plasmid DNA. The complexes were mixed with 5×10^5 cells and were seeded into 6-well plates. After 24 h of transfection, cells were incubated for another 24 h. Cells were rinsed with ice-cold PBS and were then lysed by shaking for 15 min in reporter lysis buffer (Promega). Luciferase activity in the supernatant was analyzed using a luciferase assay kit (BioThema AB) according to the manufacturer's protocol in a Victor^2 1420 multilabel counter equipped with a dispenser. Chemiluminescence values were normalized by protein content (Bio-Rad Protein Assay, Bio-Rad).

Lifespan Analysis. N2 wild type *C. elegans* were maintained, synchronized, and transferred into liquid NGM. Survival of the nematodes at 25 °C was examined daily by their response to touching first the posterior and then the anterior part. Nonresponsive nematodes were further cut in half. *C. elegans* that were scored as alive were transferred daily into fresh incubation medium. Nematodes displaying signs of internal hatching or extruded internal organs were excluded from the analysis.

SKN-1 Translocation. Transgenic *C. elegans* LD001 were maintained, synchronized, and transferred into liquid NGM. They were incubated for 1 h, then mounted onto microscope slides, and

anesthetized using 10 mM NaN_3 before analysis. Thirty nematodes showing GFP fluorescent nuclei in the intestinal cells were counted.

Statistical Analysis. Results are expressed as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 5 software (La Jolla, CA, USA). The minimum level of significance was $p < 0.05$. Statistical significance was assessed by an unpaired Student's t test with two-sided testing. When appropriate, one-way ANOVA with Dunnet's post-test or two-way ANOVA with Bonferroni post-tests was used. Lifespan analysis was performed using Kaplan–Meier statistics. Nematodes that were lost, showed internal hatching, or were killed during handling were discriminated.

RESULTS

Antioxidative Capacity of Baicalein. The antioxidative capacity of baicalein was investigated in different assay systems (Figure 2). The antioxidant capacity of baicalein in cell-free systems was demonstrated in the Trolox equivalent antioxidative capacity (TEAC) assay. In this assay the ability of a compound to reduce and thereby decolorize a green radical solution is compared to the effects of the synthetic vitamin E derivative Trolox. Compared to this reference compound, baicalein possesses an even higher radical scavenging potential already at a concentration of 5 μM (Figure 2a). The effect of baicalein on the intracellular accumulation of reactive oxygen species (ROS) in Hct116 cells was analyzed using the fluorescent probe DCF. Incubation of Hct116 cells with baicalein (4 h) did not have any effects on the unstimulated (= physiological) ROS levels, whereas incubation with 500 μM H_2O_2 (1 h) resulted in a high increase in DCF fluorescence (Figure 2b). A preincubation with baicalein (4 h) before H_2O_2 treatment significantly lowered the intracellular ROS levels in Hct116 cells. A similar effect was observed in *C. elegans* that had been pretreated with baicalein for 2 days compared to DMSO-treated control group. Baicalein treatment significantly lowered the increase in DCF fluorescence caused by thermal stress in this model organism (Figure 2c).

Activation of the Nrf2/ARE Pathway by Baicalein. For activation of the Nrf2/ARE pathway, a translocation of the transcription factor Nrf2 into the nucleus is required. This was investigated using a Nrf2-GFP fusion protein: Hct116 cells were transiently transfected with a human Nrf2::GFP construct and incubated with baicalein (40 μM) for 4 h. Fluorescence microscopy revealed a Nrf2 distribution over the entire cells with an increased concentration in the nuclei (Figure 3). The fluorescence intensity of baicalein-treated cells is much brighter compared to control cells. To further elucidate the effects of baicalein on the localization of Nrf2, nuclear and cytosolic fractions were isolated and subjected to Western blot analysis after incubation with different concentrations of this flavonoid. A concentration-dependent accumulation of Nrf2 in the nuclear fraction was detected (Figure 4a), whereas constantly only marginal levels were detectable in the cytosolic fraction. Consistently, a concentration-dependent increase in Nrf2 was detected in the total protein fraction of baicalein-treated cells (Figure 4b). The nuclear localization of Nrf2 increases the transcriptional activity of the ARE, as determined using a luciferase assay. Incubation with baicalein (50 μM) for 24 h results in an approximately 3-fold increase in luciferase activity (Figure 5a). Additionally, baicalein increases the expression of the Nrf2 target gene HO-1: Application of 40 μM baicalein results in a time-dependent increase of HO-1 protein from about 2-fold (10 h) to 3-fold basal levels after 24 h (Figure 5b,c). These results clearly indicate an activation of the Nrf2-ARE signaling pathway by the flavonoid baicalein in Hct116 cells.

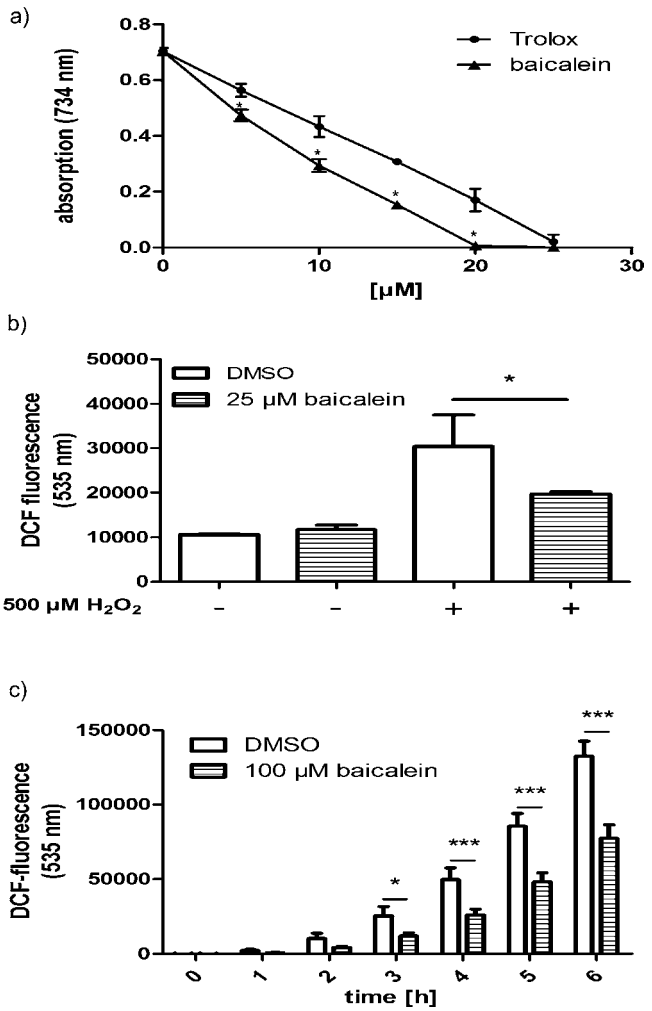

Figure 2. Antioxidative effects of baicalein. (a) Antioxidative capacity of baicalein in a cell-free system (TEAC). Decrease of absorption (734 nm) correlates with ABTS radical scavenging activity; data are the mean ± SD, $n = 3$; (*) $p < 0.01$ versus corresponding Trolox concentration. (b) Intracellular ROS accumulation in Hct116 cells was investigated using the fluorescent probe H_2DCF-DA. The cells were first incubated with baicalein for 4 h followed by incubation with H_2O_2 for 1 h, and then fluorescence of DCF as a marker of intracellular generation of ROS was measured by flow cytometry; data are the mean ± SD, $n = 3$; (*) $p < 0.05$ versus corresponding DMSO value. (c) In vivo DCF assay: influence of baicalein on ROS accumulation in wild type *C. elegans*. Nematodes were incubated with baicalein for 2 days and were then subjected to thermal stress (37 °C); DCF fluorescence intensity correlates with intracellular ROS concentration; data are the mean ± SD, $n = 3$ with 16 individuals per group; (*) $p < 0.05$ and (***) $p < 0.001$ versus corresponding DMSO-treated group.

Baicalein Activates SKN-1 Signaling and Extends *C. elegans* Lifespan. To analyze if baicalein also activates the Nrf2 signaling pathway in the model organism *C. elegans*, we used a transgenic strain expressing a fusion protein of GFP and the Nrf2 homologue in *C. elegans* named SKN-1. Comparable to the experiments performed with the colon carcinoma cells, the activation of the Nrf2 pathway was investigated by analyzing whether diffuse or nuclear GFP distribution of this fusion protein is detectable in the fluorescence microscope. Incubation of the transgenic *C. elegans* strain with baicalein

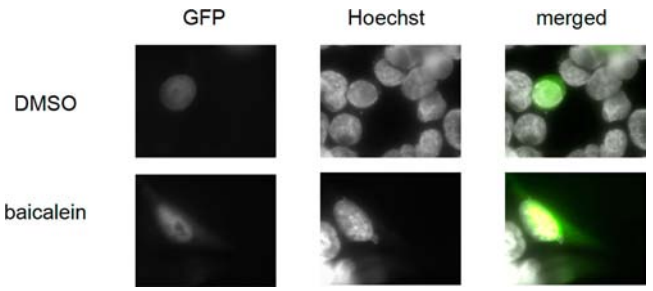

Figure 3. Baicalein modulates intracellular localization of Nrf2. Hct116 cells transfected with a Nrf2::GFP construct are stained with Hoechst 33342 and incubated with 40 μM baicalein or corresponding vehicle control for 4 h. Representative images showing GFP or Hoechst 33342 fluorescence are presented.

(100 μM) resulted in a clear increase of the fraction of nematodes that showed GFP fluorescence in the nuclei of the intestinal cells. As shown in Figure 6a, nematodes treated with vehicle DMSO show a diffuse GFP fluorescence, whereas treatment with baicalein caused an accumulation of SKN-1::GFP preferentially in the nuclei of the intestinal cells of *C. elegans* (Figure 6b). These results show clearly that baicalein activates the Nrf2 signaling pathway also in *C. elegans*: Baicalein-treated nematodes show a 5-fold higher activation of SKN-1 signaling pathway compared with the DMSO-treated control (Figure 6c). Because it is known that SKN-1 activation mediates life prolongation in *C. elegans*, we performed a lifespan assay with this model organism. Treatment of *C. elegans* with baicalein (100 μM) resulted in prolongation of the mean, median, and maximum lifespans versus the vehicle-treated control group by 45, 57, and 24%, respectively (Figure 7). These findings suggest that baicalein enhances the lifespan of *C. elegans* by activation of the SKN-1 signaling pathway.

DISCUSSION

Because certain food compounds may have important effects on intestinal signaling, we analyzed the effects of baicalein, a constituent of *S. baicalensis*, on the modulation of oxidative stress and the Nrf2 redox-active signaling pathway. The experiments were performed in both Hct116 cells and *C. elegans* to compare results from cell cultures to those from the model organism. Baicalein did not influence the basal ROS levels in Hct116 cells or *C. elegans*, but did significantly lower the ROS levels under stress conditions. Similar antioxidant effects are widespread in the vast group of flavonoids and have been shown in cell culture systems, for example, for quercetin, but are not to be generalized.[14] For certain flavonoids, for example, quercetin, antioxidative effects have also been shown in *C. elegans*.[19]

Antioxidant effects can be mediated by different mechanisms. Our results from the TEAC assay show that baicalein possesses a high radical scavenging potential. Apart from this direct antioxidative reactivity, compounds may also activate intracellular signaling pathways such as the Nrf2/ARE pathway, thereby prolonging the cellular defense reaction by up-regulating antioxidant enzymes.[20] Our results clearly demonstrate that baicalein is an activator of the Nrf2/ARE pathway in Hct116 cells. Similar findings have been published for hepatoma cells.[21] We showed that incubation of Hct116 with baicalein leads to an enhanced accumulation of the transcription factor in the nuclei by (i) fluorescence microscopy of cells expressing Nrf2::GFP and (ii) Western blot analysis of the nuclear protein fractions. Western blot analysis of the cytosolic

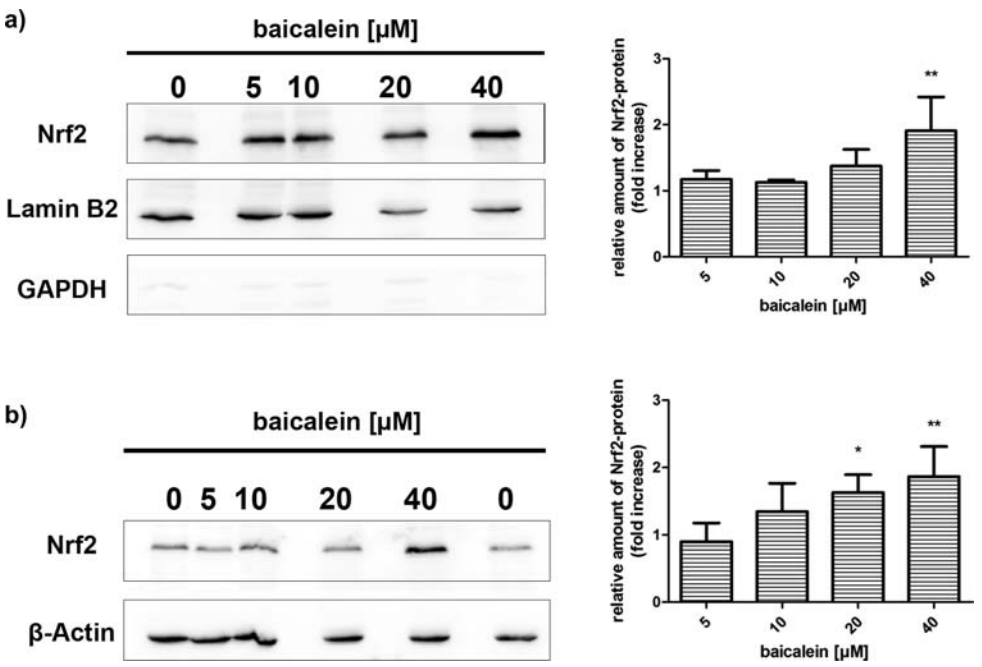

Figure 4. Baicalein mediates nuclear translocation of Nrf2-protein. (a) Western blot analysis of the nuclear fraction. Cells were incubated with different concentrations of baicalein for 4 h. Antibodies against Lamin B2 (nuclear marker) and GAPDH (cytosolic marker) were used as control for the quality of the fractionation process. One representative blot of three is shown; data (mean ± SD) are given as fold increase of Nrf2 protein amount compared to the vehicle control; (**) $p < 0.01$. (b) Western blot analysis of Hct116 total protein after incubation with different concentrations of baicalein for 4 h. β-Actin was used as a loading control. One representative blot of four is shown; data (mean ± SD) are given as fold increase of Nrf2 protein amount compared to the vehicle control; (*) $p < 0.05$ and (**) $p < 0.01$.

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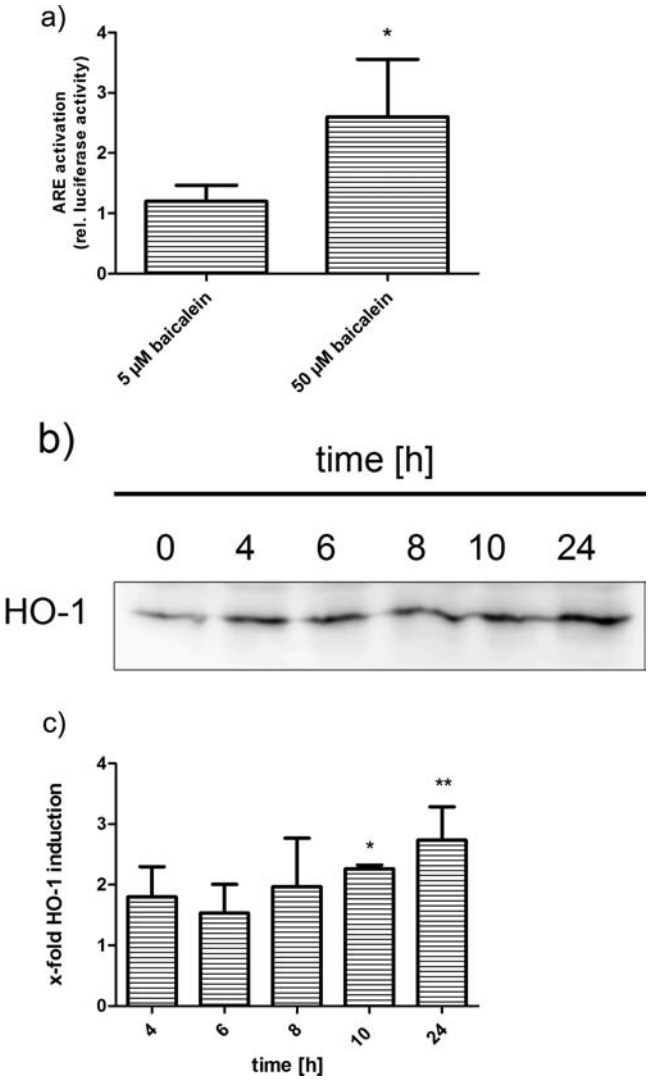

Figure 5. Baicalein activates Nrf2/ARE signaling pathway: (a) Hct116 cells are transfected with an ARE-luciferase construct and incubated with different concentrations of baicalein for 24 h. Luciferase activity is shown; data are the mean ± SD, $n = 3$; (***) $p < 0.001$ versus DMSO-treated control. (b) Time-dependent expression of HO-1 after incubation with 40 μM baicalein (Western blot analysis). One representative blot of three is shown. (c) Densitometric evaluation of time-dependent expression of HO-1 (= b). Data (mean ± SD) are given as fold increase of HO-1 protein compared to the expression at 0 h; (*) $p < 0.05$ versus 0 h and (**) $p < 0.01$ versus 0 h.

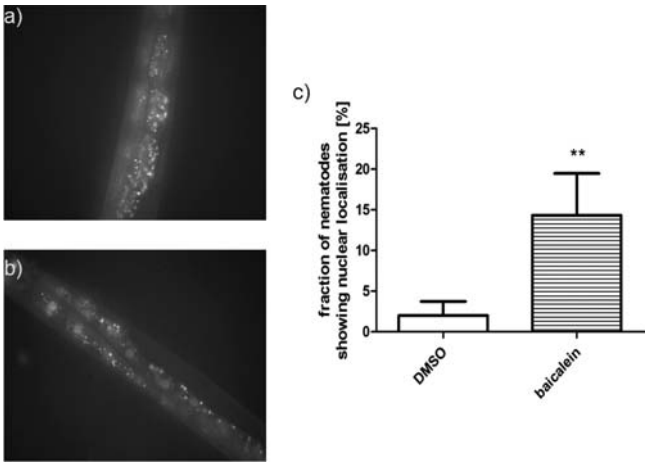

Figure 6. Baicalein induces translocation of SKN-1::GFP into nuclei of intestinal cells: 3-day-old synchronized LD001 *C. elegans* were treated with vehicle control (a) or 100 μM baicalein (b) for 1 h at 20 °C and then analyzed by fluorescence microscopy concerning visibility of GFP-fluorescence in nuclei of the intestinal cells as observed. (c) The fraction of nematodes showing nuclear GFP localization was determined: mean ± SD, 30 individuals per group in each of the three independent experiments; (**) $p < 0.01$ versus DMSO-treated control group.

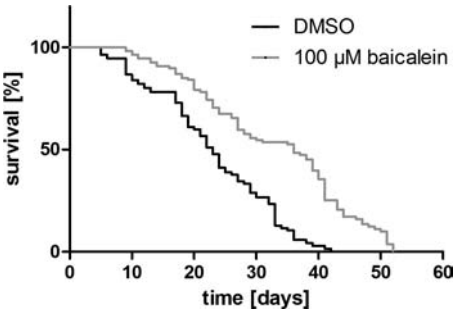

Figure 7. Baicalein prolongs the lifespan of *C. elegans*: synchronized wild type *C. elegans* were treated with 100 μM baicalein or vehicle starting at day 3 after egg laying. The nematodes were kept at 25 °C, and survival was determined daily by touch-provoked movement. Kaplan–Meier statistics were performed with a total of 120 individuals per group in three independent experiments.

protein fraction showed no alteration compared to the DMSO-treated control. Experiments with Hct116 cells expressing Nrf2::GFP display that only marginal GFP fluorescence remains in the cytosol of baicalein-treated cells compared to DMSO-treated cells. These results are consistent with the Western blot analysis of total protein extracts showing that baicalein incubation increased total cellular Nrf2 content. Therefore, we conclude that baicalein not only leads to translocation of the transcription factor from cytosol to nucleus but also has an impact on the expression or degradation of the Nrf2 protein itself by regulating the cellular amount of this protein.

Activation of the Nrf2 signaling pathway by polyphenols can be mediated by different mechanisms: On the one hand, flavonoids may induce the Nrf2 signaling pathway by formation of ROS such as H_2O_2, for example, via redox cycling.[22] On the other hand, a direct interaction of compounds with Keap1 is known[23] by which the Nrf2 pathway is activated. Because application of baicalein did not change the basal ROS level in our experimental system, it seems probable that baicalein may interact with Keap1 and not via induction of oxidative stress. We further investigated whether the translocation of Nrf2 also leads to transcriptional activation in Hct116 cells; therefore, we used an ARE-dependent luciferase vector. Incubation with baicalein increased reporter gene activity approximately 3-fold. Consistently, the expression of HO-1, which is known to be dependent on Nrf2/ARE signaling, was approximately 3-fold higher compared to basal levels. This demonstrates that the Nrf2/ARE activation by baicalein has biologically relevant effects. A slight induction of HO-1 by baicalein has previously been observed in HepG2 cells;[21] other flavonoids, for example, quercetin, have also been reported to cause a modulation of HO-1.[14] The activation of Nrf2 has been shown in cellular

systems for other polyphenols such as curcumin and epigallocatechin-3-gallate.[24]

Baicalein also activates the Nrf2 signaling pathway in the model organism *C. elegans*. We examined this using a transgenic strain expressing a SKN-1::GFP protein. Incubation with baicalein results in accumulation of GFP fluorescence in the nuclei, especially of the intestinal cells, proving a clear impact of baicalein on SNK-1 signaling. It has been demonstrated in different model organisms that even relatively small increases of active Nrf2 are biologically relevant.[25] Life prolongation was described as a prominent effect of Nrf2 activation in *C. elegans*, *Drosophila melanogaster*, and mice.[11] We analyzed the effects of baicalein on *C. elegans* lifespan. Baicalein treatment prolonged the mean, median, and maximum lifespans by 45, 57, and 24%, respectively. Similar effects have been reported for baicalein in solidified agar[27] and are also known for other specific polyphenols such as quercetin[28] and curcumin.[26] Because oxidative damage of macromolecules is postulated as a major reason for the aging process, reduction of oxidative stress and an increased presence of antioxidants are likely to correlate with longevity. Several antioxidative polyphenols that prolong the lifespan of *C. elegans* have already been identified.[26,29−32] Especially the activation of Nrf2/SKN-1 signaling increases the expression of antioxidant proteins.[11] Because basal ROS levels in *C. elegans* are not changed by baicalein treatment in the in vivo DCF assay, it seems likely that aging-related signaling pathways have to be activated in the model organism. Besides prolongation of lifespan, lowered ROS levels have also been linked to activity of stress signaling networks in *C. elegans*.[33] On the basis of our results we suggest that the Nrf2 homologue SKN-1 plays a prominent role in the baicalein-mediated prolongation of lifespan in *C. elegans*. Other pathways linked to longevity will be analyzed to further investigate molecular pathways involved in baicalein-mediated extension of lifespans.

The flavonoid baicalein activates the Nrf2/SKN-1 signaling network both in Hct116 colon carcinoma cells and in *C. elegans*. Congruent with this, incubation with baicalein reduces ROS accumulation under stress conditions in both systems. Furthermore, baicalein increases the lifespan of *C. elegans*, probably by activation of the SKN-1 signaling pathway. Our results in mammalian cells and *C. elegans* demonstrate that not simply antioxidative activity of flavonoids but interaction with intracellular signaling pathways is likely to mediate their physiological effects.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ARE, antioxidant responsive element; DCF, 2,7-dichlorodihydrofluorescein diacetate; HO-1, heme oxygenase-1; NGM, nematode growth medium; ROS, reactive oxygen species; TEAC, Trolox equivalent antioxidant capacity

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