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ORIGINAL ARTICLE

Curcumin-mediated oxidative stress resistance in *Caenorhabditis elegans* is modulated by *age-1*, *akt-1*, *pdk-1*, *osr-1*, *unc-43*, *sek-1*, *skn-1*, *sir-2.1*, and *mev-1*

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Abstract

Curcumin (diferuloylmethane), a pharmacologically active substance derived from turmeric, exhibits anti-inflammatory, anticarcinogenic, and antioxidant properties. We examined the modulation of oxidative-stress resistance and associated regulatory mechanisms by curcumin in a *Caenorhabditis elegans* model. Our results showed that curcumin-treated wild-type *C. elegans* exhibited increased survival during juglone-induced oxidative stress compared with the control treatment. In addition, curcumin reduced the levels of intracellular reactive oxygen species in *C. elegans*. Moreover, curcumin induced the expression of the *gst-4* and *hsp-16.2* stress response genes. Lastly, our findings from the mechanistic study in this investigation suggest that the antioxidative effect of curcumin is mediated via regulation of *age-1*, *akt-1*, *pdk-1*, *osr-1*, *unc-43*, *sek-1*, *skn-1*, *sir-2.1*, and *mev-1*. Our study elucidates the diverse modes of action and signaling pathways that underlie the antioxidant activity exhibited by curcumin in vivo.

Keywords: curcumin, oxidative stress, signaling pathway, *C. elegans*

Introduction

Turmeric has long been used throughout Asia as a food additive and a traditional herbal medicine. Curcumin has garnered attention in Western medicine due to its non-steroidal anti-inflammatory and chemopreventive properties. Curcumin (diferuloylmethane), a yellow coloring agent that is present in the spice turmeric (*Curcuma longa*) and that belongs to the ginger (Zingiberaceae) family, is a pharmacologically active derivative of turmeric. Traditional Indian medicine considers curcumin a drug that is applicable for the treatment of several disorders including upset stomach, flatulence, dysentery, ulcers, jaundice, arthritis, sprains, wounds, acne, and skin and eye infections [1]. Recently, many lines of evidence indicate that curcumin exhibits anti-inflammatory, anticarcinogenic, and antioxidant properties [2–4]. In particular, curcumin can inhibit tumor formation, promotion, progression, and dissemination in animal models, suggesting that curcumin represents a potential tool for cancer therapy [5]. Recently, a number of preclinical and clinical studies have indicated that curcumin exhibits potential therapeutic value against several chronic diseases including neoplastic, neurological, cardiovascular, pulmonary, metabolic, and psychological diseases [6].

Oxidative stress is considered a predominant factor in the pathophysiology of various diseases and aging [7], occurring as a result of excessive generation of reactive oxygen species (ROS) or diminished antioxidative defense systems. Curcumin has been reported to represent a potent inhibitor of ROS formation [8–9]. However, curcumin not only represents a simple antioxidant but also acts as an electrophilic compound that can trigger the Nrf2/ARE signaling pathway, which plays a key role in activating antioxidative enzymes, Phase-II enzymes, heme oxygenase, HSP70, and thioredoxin reductase, which might exhibit a pivotal role in oxidative stress-induced diseases [10].

Curcumin has been shown to elicit a variety of beneficial human health effects. However, the mechanisms by which curcumin acts remain to be further elucidated [11]. Previously, many key findings with relevance to mammals were discovered using the well characterized *C. elegans* model. This has been possible because of the strong biological conservation between *C. elegans* and mammals, as the *C. elegans* homologs of 60–80% of human genes have been identified [12–13]. *C. elegans* is a model that has been increasingly used to study the effects of pharmacologically active compounds of herbal origin on biological processes, as well as to identify new targets for pharmacological interventions [13–18]. In this study, we investigated the antioxidative potentials and associated regulatory mechanisms underlying the effects of curcumin in *C. elegans*. The influences of curcumin on the expression of the antioxidant enzymes superoxide dismutase (SOD), glutathione S-transferase (GST), and heat-shock proteins (HSPs) were also examined. Moreover, we dissected the genetic requirements that are attributable to the increased oxidative stress resistance resulting from treatment with curcumin.

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Methods and materials

Chemicals, Caenorhabditis elegans strains, and handling procedures

All of the chemicals used in this study were obtained from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA) unless otherwise stated. The curcumin was dissolved in DMSO (dimethylsulfoxide). A final DMSO concentration of 0.1% (v/v) was maintained in all of the conditions.

The following *C. elegans* strains were used in this study: Bristol N2 (wild-type); TK22, *mev-1* (*kn1*); CB1370, *daf-2* (*e1370*); TJ1052, *age-1* (*hx546*); GR1310, *akt-1* (*mg144*); GR1318, *pdk-1* (*mg142*); GR1307, *daf-16* (*mgDf50*); AM1, *osr-1* (*rm1*); MT2605, *unc-43*- (*n498n1186*); AU1, *sek-1* (*ag1*); EU1, *skn-1* (*zu67*); VC199, *sir-2.1* (*ok434*); FK171, *mek-1* (*ks54*); CF1553, muIs84 [pAD76 (*sod-3*::GFP)]; CL2166, dvIs19 [pAF15 (*gst-4*:: GFP::NLS)]; and CL2070, dvIs70 [*hsp-16.2*::GFP; *rol-6* (*su1006*)]. Before our analysis, the *sir-2.1* (*ok434*) strain was backcrossed twice against the WT strain. All of the *C. elegans* strains, as well as the *Escherichia coli* OP50 strain, were obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota, USA. The worms were maintained (unless otherwise stated) at 20°C on nematode growth medium (NGM) agar plates carrying a lawn of *E. coli* OP50 as previously described [19]. The synchronization of the worm cultures was achieved by the hypochlorite treatment of gravid hermaphrodites [20].

Oxidative stress assays

Synchronized L1 larvae were incubated in liquid S-basal containing 10^9 cells/ml of *E. coli* OP50 bacteria and either curcumin or 0.1% DMSO as the solvent control for 72 h at 20°C. Subsequently, the worms were immediately subjected to oxidative stress. Juglone (5-hydroxy-1, 4-naphthoquinone), a ROS-generating compound, was used to induce oxidative stress in worms. Curcumin (10, 20, 100, and 200 μM) or control (0 μM)-treated worms were transferred to S-basal medium containing juglone at 20°C and then scored for viability. The test was performed independently at least three times. Approximately 60–80 worms were scored in each experiment. Due to the variable sensitivity of the mutant strains, juglone exposure conditions were slightly modified as follows: the WT N2, *mev-1*, *daf-2*, *age-1*, *akt-1*, *pdk-1 daf-16*, *unc-43*, *sek-1*, *skn-1*, *sir-2.1*, and *mek-1* mutant worms were exposed to 250 μM juglone; the *osr-1* mutant worms were exposed to 50 μM juglone. The survival of the worms was determined by touch-provoked movement [21]. The worms were scored as dead when they failed to respond to repeated touching with a platinum wire pick [21].

Intracellular ROS measurement

Synchronized L1 larvae were incubated in liquid S-basal medium containing 10^9 cells/ml *E. coli* OP50 bacteria and either curcumin or 0.1% DMSO as the solvent control (0 μM) for 72 h at 20°C. Subsequently, we measured the intracellular ROS levels in the curcumin- and control-treated worms using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA). One hundred and fifty nematodes were homogenized by sonication after each treatment, and the worm lysates were collected for the ROS measurement [22–23]. The worm samples were incubated with H_2DCFDA (at a final concentration of 50 μM in phosphate-buffered saline) for 2.5 h at 20°C, and the resulting fluorescence was quantitated using a FLx800 Microplate Fluorescent Reader (Bio-Tek Instruments, Winookski, VT, USA) at excitation and emission wavelengths of 485 and 530 nm, respectively. At least three independent experiments were performed.

Induction of a stress-response reporter

Synchronized L1 larvae containing an inducible green fluorescent protein (GFP) reporter for *gst-4*, *sod-3*, and *hsp-16.2* were incubated in liquid S-basal medium containing 10^9 cells/mL of *E. coli* OP50 bacteria and a final concentration of either 20 μM curcumin or 0.1% DMSO as the solvent control for 72 h. We next directly measured the expression of the *sod-3*, *gst-4*, and *hsp-16.2* genes by quantitating the fluorescence of the GFP reporter.

Approximately 30 randomly selected worms from each set of experiments were mounted onto microscope slides that were coated with 3% agarose, anesthetized with 2% sodium azide, and mounted with coverslips. Epifluorescence images were captured using a Leica epifluorescence microscope (Leica, Wetzlar, Germany) equipped with a filter set (excitation at 480 ± 20 nm; emission at 510 ± 20 nm) and a cooled charge-coupled device camera. The adult worms were examined, and the total GFP fluorescence of each whole worm was quantified using Image-Pro Plus software (Media Cybernetics, Bethesda, MD, USA).

Data analysis

For the oxidative stress assay, median lethal time (LT_{50}) was determined using Probit (USEPA), and the survival curves were analyzed by log-rank test using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). The survival curves with p values < 0.05 relative to the control curves were considered significantly different. Statistical analysis was performed using SPSS Statistics 17.0 Software (SPSS, Inc., Chicago, IL., 2008). The results are presented as the mean values ± standard error (SE). We tested the statistical significance of the differences between the populations by one-way analysis of variance (ANOVA) and the least significant difference (LSD) post-hoc test. The differences were considered statistically significant when $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

Results

Curcumin enhances oxidative-stress resistance in C. elegans

To investigate whether curcumin elicits an antioxidant effect in *C. elegans*, WT N2 worms were pretreated with curcumin, followed by exposure to juglone-induced oxidative stress. WT N2 synchronized L1 larvae were pretreated with 10, 20, 100, and 200 μM curcumin or 0.1% DMSO as the solvent control for 72 h at 20°C. The adult animals were then exposed to 250 μM juglone, a redox cycler that generates intracellular oxidative stress [24–26]. Our results indicated that pretreatment with 10, 20, 100, and 200 μM curcumin significantly increased the survival of worms in a curcumin concentration-dependent manner that were exposed to juglone-induced oxidative stress (Figure 1).

Curcumin reduces intracellular ROS in C. elegans

To determine whether curcumin-enhanced oxidative-stress resistance was attributed to its ROS-scavenging ability, we measured the intracellular ROS levels in the worms. Non-fluorescent DCF-DA is a freely cell-permeable dye, which is readily converted to fluorescent 2′7′-dichlorofluorescein (DCF) via an interaction with intracellular H_2O_2. As shown in Figure 2A, the intracellular ROS levels were significantly decreased when the WT N2 worms were treated with 10, 20, 100, and 200 μM curcumin. As the antioxidant effect and the intracellular ROS levels were significantly enhanced and reduced, respectively, at concentrations of curcumin as low as 20 μM, we chose 20 μM curcumin as the working concentration for subsequent experiments.

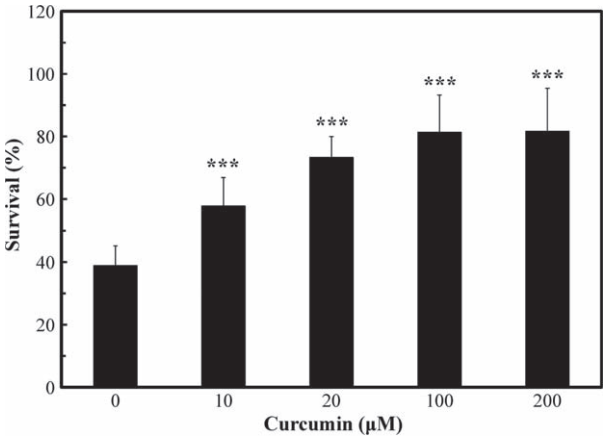

Figure 1. The effects of curcumin on the oxidative stress resistance of WT *C. elegans* N2. Synchronized WT L1 larvae were pretreated with curcumin (10, 20, 100, and 200 μM) or 0.1% DMSO as the solvent control for 72 h at 20°C. Subsequently, the adult worms were immediately subjected to oxidative stress assays. For the oxidative stress assays, curcumin- and control-treated (0.1% DMSO, 0 μM) adult worms were exposed to 250 μM juglone for 3 h at 20°C and then scored for viability. The experiment was performed independently at least 3 times. Approximately 60–80 worms were scored in each experiment. The results presented are the mean values ± SE. Differences compared with the control (0 μM, 0.1% DMSO) were considered statistically significant when $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) by one-way ANOVA and the LSD post-hoc test.

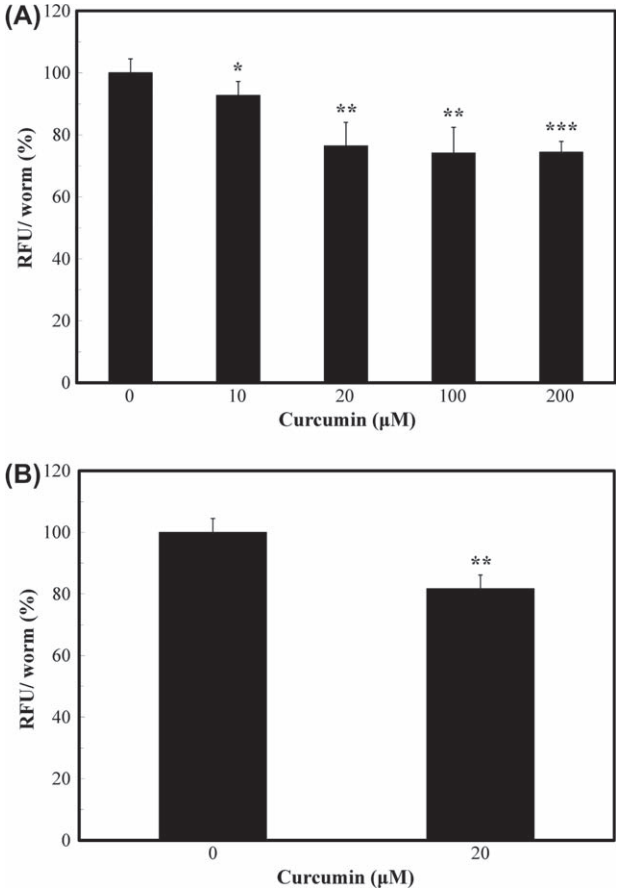

Figure 2. The effects of curcumin on the accumulation of intracellular ROS in *C. elegans*. Synchronized WT (A) or TK22 (*mev-1*) mutant (B) L1 larvae were pretreated with curcumin (10, 20, 100, and 200 μM for WT N2; 20 μM for TK22 (*mev-1*)) or 0.1% DMSO (0 μM) as the solvent control for 72 h at 20°C. Subsequently, intracellular ROS was measured using 2′,7′-dichlorodihydrofluorescein diacetate. One hundred and fifty worms from each condition were used to analyze the intracellular ROS levels. The results are presented as relative fluorescence units of an individual worm. The experiment was performed independently at least 3 times. The results are presented as the mean ± SE. Differences compared with the control (0 μM, 0.1% DMSO) were considered statistically significant when $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) by one-way ANOVA and the LSD post-hoc test.

We further evaluated the free radical scavenging abilities of curcumin using oxidative stress-hypersensitive *mev-1* mutant worms. MEV-1 is a subunit of the succinate-coenzyme Q oxidoreductase in Complex II of the electron transport chain [27], and *mev-1* mutant worms are hypersensitive to oxidative stress, which is predominantly attributed to overproduction of mitochondrial ROS [27]. Synchronized L1 *mev-1* mutant larvae were pretreated with 20 μM curcumin for 72 h at 20°C. Subsequently, we directly prepared the *mev-1* mutant worms for intracellular ROS measurement. Figure 2B shows that 20 μM curcumin significantly attenuated the ROS

overproduction in *mev-1* mutant worms compared with the untreated control-treated *mev-1* mutant worms ($p < 0.01$, Figure 2B). Taken together, curcumin might act against juglone-induced oxidative stress via its intracellular ROS-scavenging ability and decrease mitochondrial ROS toxicity.

Curcumin enhances the expression of gst-4 and hsp-16.2 but not of sod-3 in C. elegans

To elucidate whether the increased oxidative-stress resistance detailed above was attributed to curcumin-induced alterations in the regulation of a specific stress-response gene, we examined the responsiveness of the antioxidant enzymes SOD, GST, and HSPs. SOD is a predominant enzyme that protects against oxidative stress by catalyzing the removal of O_2^- [28]. *C. elegans* manganese SOD, SOD-3, is an antioxidant enzyme that is induced in response to stress [29]. The enzymes of the GST family are involved in the Phase-II detoxification process, and *C. elegans* GST-4 is involved in the oxidative-stress response [30]. HSPs are highly conserved stress response proteins that are induced by thermal or oxidative stresses. The HSP-16.2 family of proteins is homologous to αB crystalline, and its members are expressed under stress conditions in *C. elegans* [31].

Transgenic *C. elegans* (CF1553, CL2166, and CL2070) expressing GFP as a reporter transgene for the inducible expression of *sod-3*, *gst-4*, *and hsp-16.2* were treated with 20 μM curcumin or 0.1% DMSO as the solvent control for 72 h at 20°C. Subsequently, we directly measured the expression levels of *sod-3*, *gst-4*, *and hsp-16.2* by quantitating the fluorescence of the GFP reporter. Figure 3A shows representative images of transgenic worms that were exposed to curcumin. Our results show that the expression levels of *gst-4* and *hsp-16.2* were

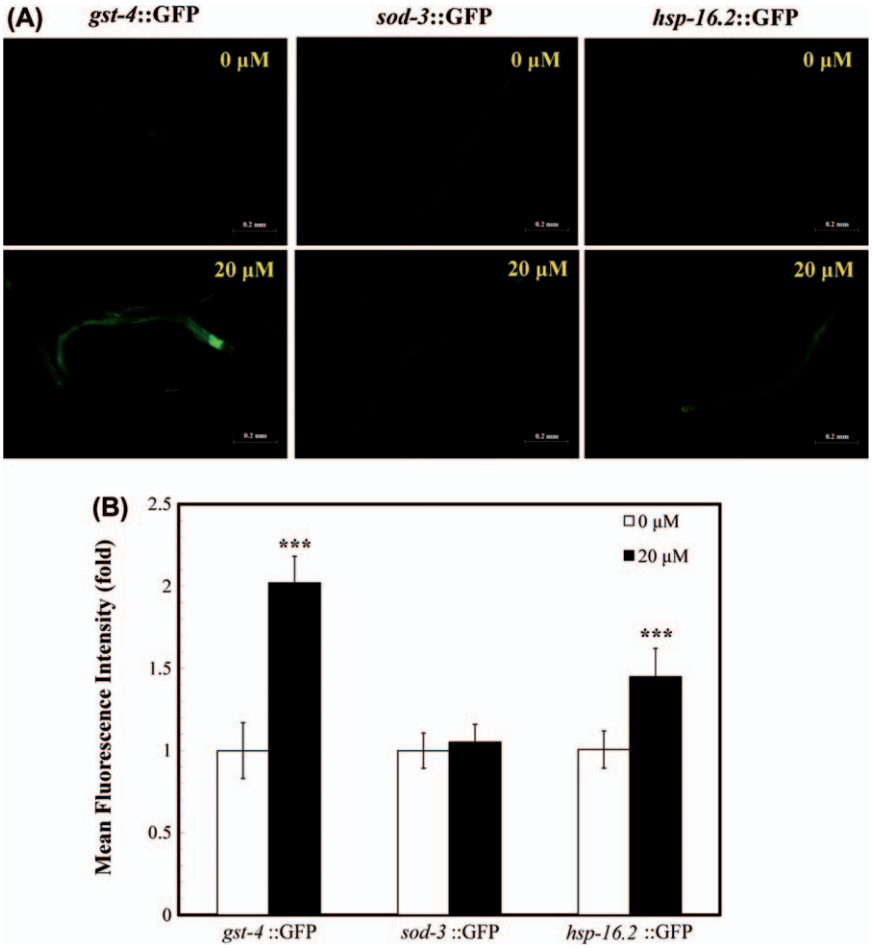

Figure 3. The effects of curcumin on the expressions of GST-4, SOD-3, and heat shock protein (HSP-16.2) in *C. elegans*. Immediately after hatching, age-synchronized, transgenic worms of the CF1553 strain (*sod-3*::GFP), CL2166 strain (*gst-4*::GFP), and CL2070 strain (*hsp-16.2*::GFP) were incubated with 20 μM curcumin or 0.1% DMSO as the solvent control in liquid S-basal medium for 72 h. (A) Representative fluorescence images of curcumin- and control-treated stress response worm strains. (B) The relative fluorescence intensities in transgenic worms. Total GFP fluorescence for each whole worm was quantified using Image-Pro Plus software. Relative fluorescent intensities were calculated by normalizing the average GFP intensities of each treatment condition to that of the control treatment group (0 μM curcumin). Approximately 30 randomly selected worms from each set of experiments were examined. The experiment was performed independently 3 times. The results are presented as the mean values ± SE. Differences compared with the control (0 μM, 0.1% DMSO) were considered statistically significant when $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) by one-way ANOVA and the LSD post-hoc test.

significantly upregulated by 20 μM curcumin treatment compared to the control treatment, whereas curcumin did not upregulate the expression of *sod-3* (Figure 3B), suggesting that curcumin-mediated oxidative stress resistance in *C. elegans* is not only mediated by the induction of antioxidative enzymes but potentially by more complex mechanisms.

Genetic requirements for oxidative stress resistance from curcumin treatment

To elucidate the importance of genes that are relevant to the oxidative stress resistance conferred by curcumin, we examined the ability of loss-of-function mutants to increase their oxidative stress resistance during curcumin treatment. The premise of these experiments was that curcumin treatment would not increase the survival of mutant nematodes lacking specific genes that are required for the antioxidant effects elicited by curcumin during juglone-induced oxidative stress. Considerable evidence has shown that several stress-response pathways can affect the stress resistance of *C. elegans* [32–35]. Therefore, we evaluated select genes for their involvement in curcumin-mediated oxidative stress resistance in *C. elegans*. Synchronized L1 mutant larvae were pretreated with 20 μM curcumin or 0.1% DMSO for 72 h at 20°C followed by treatment with juglone. During pretreatment with 20 μM curcumin, no adverse effects on the mutant worms, including the survival, growth rate, progeny production, body length, or morphological changes were observed.

Our results show that curcumin treatment significantly increased the survival of the *daf-2*, *daf-16*, and *mek-1* mutants, suggesting that curcumin might act independently of the *daf-2*, *daf-16* and *mek-1* genes (Table I and Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779). In addition, curcumin treatment increased the survival of the *mev-1* mutant worms (Table I and Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779), indicating that curcumin can improve survival during oxidative stress conditions. In contrast, we did not observe significantly enhanced oxidative stress resistance in the *age-1*, *akt-1*, *pdk-1*, *osr-1*, *unc-43*, *sek-1*, *skn-1*, or *sir-2.1* mutant worms (Table I and Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779), suggesting that those genes are required for curcumin-mediated oxidative stress resistance in *C. elegans*.

Table I. Effects of curcumin on oxidative-stress resistance of wild-type and mutant *C. elegans*.

Genotype	$LT_{50} \pm SE$ (h)		Log-rank test[a]
	Untreated	Treated	
N2	2.51 ± 0.21	3.56 ± 0.11	$P < 0.001$***
mev-1 (*kn-1*)	2.46 ± 0.06	3.13 ± 0.26	$P = 0.027$*
daf-2 (*e1370*)	7.63 ± 0.80	10.91 ± 1.47	$P = 0.0072$**
age-1 (*hx546*)	3.23 ± 0.30	3.68 ± 0.35	$P = 0.1265$
akt-1 (*mg144*)	3.62 ± 0.37	3.74 ± 0.25	$P = 0.2125$
pdk-1 (*mg142*)	4.74 ± 0.20	4.67 ± 0.08	$P = 0.9704$
daf-16 (*mgDf50*)	2.72 ± 0.10	3.59 ± 0.21	$P < 0.001$***
osr-1 (*rm1*)	1.13 ± 0.05	1.10 ± 0.01	$P = 0.9861$
unc-43 (*n498n1186*)	1.76 ± 0.02	1.68 ± 0.14	$P = 0.7326$
sek-1 (*ag1*)	1.10 ± 0.03	1.16 ± 0.03	$P = 0.486$
skn-1 (*zu67*)	0.69 ± 0.15	0.65 ± 0.09	$P = 0.4657$
sir-2.1 (*ok434*)	1.31 ± 0.08	1.22 ± 0.11	$P = 0.4434$
mek-1 (*ks54*)	1.93 ± 0.32	2.74 ± 0.15	$P < 0.001$***

[a]Statistical significance of the difference between the survival curves was demonstrated by log-rank test using the Kaplan–Meier survival analysis.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

Moreover, we further evaluated genes that are relevant to oxidative stress resistance conferred by curcumin by measuring the intracellular ROS levels in the mutant worms. Similar to what we observed in the juglone-induced oxidative stress assays, curcumin treatment significantly decreased ROS levels in the *daf-2*, *daf-16*, and *mek-1* mutant worms, similar to what was observed with the WT N2 worms (Figure 4). No significant differences in ROS levels were observed in the *age-1*, *akt-1*, *pdk-1*, *osr-1*, *unc-43*, *sek-1*, *skn-1*, and *sir-2.1* mutants (Figure 4).

Discussion

In this study, we used *C. elegans* as an in vivo model to examine the protective potential and mode of action of curcumin. Several studies have indicated that the protective actions of flavonoids and polyphenols in *C. elegans* are predominantly attributed to their antioxidative activities [15,36]. We show that the survival of WT worms was significantly increased in a curcumin concentration-dependent manner in the presence of juglone-induced oxidative stress (Figure 1), suggesting that curcumin exhibits antioxidative activity in *C. elegans*. Similar lifespan effects were observed with curcumin treatment alone [37]. In our previous study, we demonstrated that the lifespan extension induced by curcumin in *C. elegans* is attributed to its antioxidative properties [37]. Moreover, we showed that lifespan extension elicited effects on body size and pharyngeal pumping rate but not on reproduction [37].

Elevated ROS generation likely represents an important feature of oxidative stress-related toxicity. Figure 2A shows that curcumin significantly decreased the intracellular ROS levels of WT *C. elegans*. Furthermore, curcumin enhanced the survival and decreased the intracellular ROS levels in *mev-1* mutant worms (Table I, Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779, and Figure 2B), which is an ROS-generating strain. Our findings are consistent with previous reports [38–39]. Therefore, curcumin might act against oxidative stress due to its ROS-scavenging ability, which decreases mitochondrial ROS toxicity.

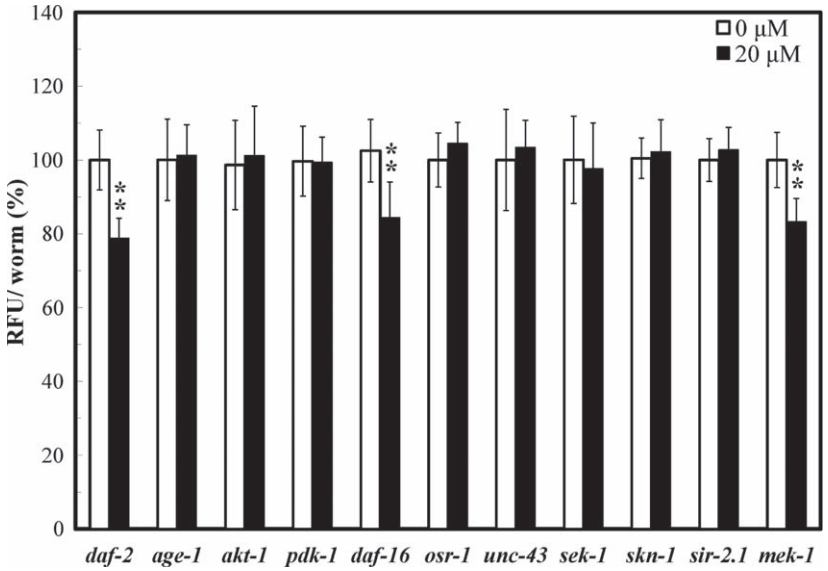

Figure 4. The effects of curcumin on the accumulation of intracellular ROS in mutants. Synchronized mutant L1 larvae were pretreated with curcumin (20 μM) or 0.1% DMSO as the solvent control for 72 h at 20°C. Subsequently, intracellular ROS was measured using 2′,7′-dichlorodihydrofluorescein diacetate. One hundred and fifty worms from each condition were used to analyze the intracellular ROS levels. The experiment was performed independently 3 times. The results are presented as the mean values ± SE. Differences compared with the control (0 μM, 0.1% DMSO) were considered statistically significant when $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) by one-way ANOVA and the LSD post-hoc test.

Previous studies have demonstrated that curcumin affects numerous intracellular systems, such as transcription factor nuclear factor κB (NFκB), hypoxia-inducible factor-1 (HIF-1), and nuclear factor erythroid 2-related factor 2 (Nrf2) [40]. However, the mechanisms underlying its activity are not well defined. In *C. elegans*, oxidative stress response is regulated by several stress response pathways, including the insulin/IGF-1 (IIS), JNK (c-Jun N-terminal kinase) MAPK, and the p38 MAPK signaling pathways [32–35]. To identify the genetic requirements for oxidative stress resistance by curcumin, we assessed the requirement of select stress response pathway genes for the antioxidant activities of curcumin.

DAF-16 (a FOXO-family transcription factor) is a transcription factor in the insulin signaling pathway, which is considered as a key regulator of many important biological processes including lifespan, metabolism, and stress responses [41]. We examined the oxidative stress resistance of loss-of-function *daf-16* deletion mutant worms during curcumin exposure. Curcumin treatment significantly increased survival and decreased intracellular ROS levels in *daf-16* mutant worms (Table I, Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779, and Figure 4). In addition, the expression levels of *sod-3*, a target gene of DAF-16, were not induced following curcumin treatment (Figure 3), suggesting that curcumin might act independently of DAF-16. Similar findings were found in a previous polyphenol quercetin-mediated stress resistance study [42]. Furthermore, curcumin significantly increased survival and decreased intracellular ROS levels in *daf-2* mutant worms, suggesting that curcumin might act independently of DAF-2 (Table I, Supplementary Figure 1 to be found online at http://informa healthcare.com/doi/abs/10.3109/10715762.2013.872779, and Figure 4). DAF-2 is the only insulin/IGF-1 like receptor in *C. elegans*. DAF-2 is key player in the IIS pathway, which inhibits DAF-16 activity.

Notably, curcumin did not enhance survival or influence the intracellular ROS levels of the long-lived and oxidative stress-resistant mutant *age-1* strains (Table I, Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779, and Figure 4), suggesting that AGE-1, a catalytic subunit of a phosphoinositide 3-kinase (PI3K), might be involved in curcumin-mediated oxidative stress resistance. Similar findings were found in a previous quercetin-mediated lifespan extension study [43]. Moreover, curcumin did not enhance the survival or affect the intracellular ROS levels in the *akt-1* and *pdk-1* mutant strains (Table I, Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779, and Figure 4), suggesting that AKT-1 and PDK-1 are at least partly involved for curcumin-mediated oxidative stress resistance and can be explained by a possible action of AKT-1 and PDK-1 that are independent of ILS. Similar findings were found in a previous catechin-mediated longevity study [44]. Furthermore, it has been reported that AKT-1 and AKT-2 negatively regulate (independent of DAF-16) DNA-damage-induced apoptosis in the *C. elegans* germline [45]. As we did not find that DAF-2 and DAF-16 were required for the oxidative stress resistance conferred by curcumin, it is possible that the ILS-pathway is not required or unidentified members of the IIS family are involved in the curcumin-mediated antioxidant activity.

In *C. elegans*, the transcription factor SKN-1 is the ortholog of mammalian Nrf proteins, which induce the Phase-II detoxification response that is protective against oxidative stress and that acts via multiple longevity pathways [46–47]. Our results show that treatment with curcumin did not enhance oxidative stress resistance or influence the intracellular ROS levels of the *skn-1* mutant strains (Table I, Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10715762.2013.872779, and Figure 4). In addition, treatment with curcumin upregulated the expression of *gst-4* (Figure 3), a target gene of SKN-1, reflecting curcumin-mediated oxidative stress resistance is via SKN-1.

Apart from the mutants detailed above, curcumin pretreatment followed by juglone exposure did not increase the survival of worms lacking the *unc-43*, *sek-1*, *osr-1*, and *sir-2.1* genes. Moreover, curcumin pretreatment did not affect the intracellular ROS levels in worms lacking the *unc-43*, *sek-1*, *osr-1*, and *sir-2.1* genes (Figure 4). Disruption of the calmodulin kinase II (CaMKII) pathway appeared to impair the antioxidative activity of curcumin. *C. elegans* OSR-1 was previously shown to regulate survival during osmotic stress via the UNC-43 (CaMKII) and SEK-1 (MAPKK in the p38 pathway) signaling cascades [48]. UNC-43 is part of a neuronal regulatory signaling pathway that is upstream of SEK-1, which facilitates resistance to pathogenic bacteria and oxidative stress by mediating the translocation of cytoplasmic DAF-16 and SKN-1 to the nuclei of intestinal cells [49–51]. Many antioxidant processes or enzymes are regulated by Ca^{2+}, and increased cellular levels of Ca^{2+} might represent a trigger for increased ROS generation [52]. The OSR-1/UNC-43/SEK-1 pathway was found to be a target for blueberry polyphenols in *C. elegans* [15]. In addition, the flavonoid quercetin-mediated lifespan extension is modulated by UNC-43 and SEK-1 [43]. Taken together, these findings indicate that the action of curcumin in *C. elegans* is likely mediated through the CaMKII and p38 kinase pathways, which increase stress resistance and longevity via the translocation of SKN-1 into the nuclei of intestinal cells. *C. elegans* SIR-2.1, a member of the Sir-2 family of NAD-dependent histone deacetylases, was identified as crucial in stress response, and the overexpression of *sir-2.1* reduces the nuclear accumulation of H_2O_2 in worms [53]. It has been shown that treatment of *C. elegans* with the small molecule resveratrol extends the lifespan in a *sir-2.1*-dependent but *daf-16*-independent manner [54].

Our previous study showed that the lifespan extension induced by curcumin in *C. elegans* is attributed to its antioxidative properties and, furthermore, that *osr-1*, *sek-1*, *mek-1*, *skn-1*, *unc-43*, *sir-2.1*, and *age-1* are required for curcumin-mediated longevity in *C. elegans* [37]. Therefore, the activity of curcumin in juglone-induced oxidative stress resistance is likely mediated via the regulation of signaling pathways, as is the lifespan extension by curcumin in *C. elegans*.

In conclusion, our study demonstrates that curcumin can increase in vivo oxidative stress resistance by attenuating the accumulation of intracellular ROS and increasing the expression of the specific stress response genes *gst-4* and *hsp-16.2*. The mechanistic study in this investigation suggests that the antioxidative effects of curcumin are mediated via the regulation of *age-1*, *akt-1*, *pdk-1*, *osr-1*, *unc-43*, *sek-1*, *skn-1*, *sir-2.1*, and *mev-1*. Our study has unraveled diverse modes of actions and signaling pathways that underlie the antioxidant activity of curcumin in vivo.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary material available online

Supplementary Figure 1.