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Caffeic acid improves cell viability and protects against DNA damage: involvement of reactive oxygen species and extracellular signal-regulated kinase

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Abstract

Hormesis is an adaptive response to a variety of oxidative stresses that renders cells resistant to harmful doses of stressing agents. Caffeic acid (CaA) is an important antioxidant that has protective effects against DNA damage caused by reactive oxygen species (ROS). However, whether CaA-induced protection is a hormetic effect remains unknown, as is the molecular mechanism that is involved. We found that a low concentration (10 μM) of CaA increased human liver L-02 cell viability, attenuated hydrogen peroxide (H_2O_2)-mediated decreases in cell viability, and decreased the extent of H_2O_2-induced DNA double-strand breaks (DSBs). In L-02 cells exposed to H_2O_2, CaA treatment reduced ROS levels, which might have played a protective role. CaA also activated the extracellular signal-regulated kinase (ERK) signal pathway in a time-dependent manner. Inhibition of ERK by its inhibitor U0126 or by its specific small interfering RNA (siRNA) blocked the CaA-induced improvement in cell viability and the protective effects against H_2O_2-mediated DNA damage. This study adds to the understanding of the antioxidant effects of CaA by identifying a novel molecular mechanism of enhanced cell viability and protection against DNA damage.

Key words: Caffeic acid; Hormesis; Antioxidants; DNA double-strand breaks; Extracellular signal-regulated kinase

Introduction

Caffeic acid (3,4-dihydroxycinnamic acid, CaA), a naturally occurring hydroxycinnamic acid derivative, is an active phenolic component of propolis extract and is also found in a wide variety of plants (1). It has biological and pharmacological properties that include antiviral, antioxidant, anti-inflammatory, anticarcinogenic, and immunomodulatory activity (1-5). Extensive evidence from both *in vitro* and *in vivo* studies suggests that CaA is an important antioxidant and has health benefits (2,4). CaA can inhibit lipoxygenase activity and suppress lipid peroxidation (2). Moreover, CaA alleviates alcohol-induced oxidative damage in the liver and kidney (6). It can also protect against UVB-induced DNA damage by suppressing the activation of interleukin-10 and mitogen-activated protein kinases (MAPKs) (1). However, the molecular mechanisms underlying the CaA-induced protective effects against DNA damage remain unclear.

The MAPK pathways transduce signals that lead to diverse cellular responses such as cell growth, differentiation, proliferation, and apoptosis (7-9). Each of the three major MAPK pathways consists of three-tiered cascades that induce a pathway comprised of phosphorylating proteins that mediate transduction pathways activated by a variety of extracellular signals and regulate the expression of specific genes (10,11). The extracellular signal-regulated kinase (ERK) pathway typically transduces growth factor signals that induce cell differentiation or proliferation, whereas cytokines and stress signals activate the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways, resulting in stress responses, growth arrest, or apoptosis (9,12). A previous study indicated that CaA regulates lipopolysaccharide (LPS)-induced oxidative stress through c-Src/ERK signaling pathways in endothelial cells (4). We therefore hypothesized that ERK signaling

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might be involved in CaA-induced protection against DNA damage.

We found that CaA activated the ERK signaling pathway by a relatively low level of reactive oxygen species (ROS), which blocked H_2O_2-induced DNA double-strand breaks (DSBs), and improved the viability of human liver cells. Our study revealed a novel mechanism of CaA-induced protection against DNA damage in liver cells, which may help identify potential targets for the antioxidant and anticarcinogenic activities of CaA.

Material and Methods

Reagents

RPMI-1640 medium, fetal bovine serum (FBS), penicillin, and streptomycin were all purchased from Gibco Life Technologies (USA). CaA (⩾99% purity), H_2O_2, and catalase were purchased from Sigma (USA). The ERK inhibitor U0126 was purchased from Cell Signaling Technology (USA). All other reagents were of analytical grade or the highest grade available.

Cells and cell culture

The human liver cell line L-02 was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (China). Cells were maintained in 5% CO_2 at 37°C in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Determination of cell viability

Cell viability was evaluated by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt] hydrolysis using a cell counting kit (CCK-8, Dojindo Molecular Technologies, Inc., Japan). Briefly, cells were seeded in 96-well plates in triplicate at a concentration of 2×10^3 per well for 24 h. The plates were treated as indicated in the figure legends. Following treatment, 10.0 μL CCK-8 solution was added to each well, and the cells were incubated for another 4 h. Absorbance at 450 nm was measured with a multiwell plate reader (Model 680, Bio-Rad, USA). Cell viability was calculated as the ratio of the absorbance of experimental and control wells, which contained only cells and medium, and is reported as a percentage.

Measurement of intracellular ROS

Intracellular ROS levels were quantified by using the DCFDA (2′,7′-dichloro fluorescein diacetate)-Cellular Reactive Oxygen Species Detection Assay Kit (Abcam, UK). DCFDA was oxidized by ROS in viable cells to 2′,7′-dichloro fluorescein (DCF), which is highly fluorescent at 530 nm. The cells were washed three times with phosphate-buffered saline (PBS). DCFDA, diluted to a final concentration of 10 μM, was added, and the cells were incubated for 30 min at 37°C in the dark. After washing three times with PBS, fluorescence was measured with a multimode microplate reader (Tecan Trading AG, Switzerland) at excitation and emission wavelengths of 488 and 525 nm, respectively. ROS level was calculated as the absorbance ratio of experimental and control cells and expressed as a percentage.

Western blots

Cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, USA), which were probed with primary antibodies (1:500 dilution) overnight at 4°C. The antibodies used were ERK and p-ERK (Cell Signaling Technology) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Sigma). Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:1000 dilution, Cell Signaling Technology) for 1 h at room temperature. The immune complexes were detected with enhanced chemiluminescence reagents (Cell Signaling Technology). Blots were quantified by densitometry and normalized against GAPDH to correct for differences in protein loading. For densitometric analyses, the bands on the blots were measured with the Eagle Eye II imaging system (Stratagene, USA).

RNA interference

Control, ERK1, and ERK2 small interfering RNAs (siRNA) were purchased from Santa Cruz Biotechnology (USA). Transfections were performed with the N-TERTM Nanoparticle siRNA Transfection System (Sigma). Briefly, 1×10^6 cells were seeded into each well of 6-well plates and cultured for 48 h. Transfection was carried out for 12 h after adding a nanoparticle formation solution containing 20 nM target siRNA to each well. The cells were then maintained in conventional cultures for 24 h before conducting further experiments.

Statistical analysis

Derived values are reported as the means with 95% confidence intervals (CIs). Student's *t*-tests and one-way analyses of variance (ANOVAs) followed by Dunnett's *t*-tests were used to assess significant differences among groups. P values <0.05 were considered statistically significant. All tests were carried out with SPSS software (version 11.5; SPSS Inc., USA).

Results

CaA attenuated H_2O_2-induced inhibition of cell viability of L-02 cells

We first determined the effects of CaA on cell viability. Human liver L-02 cells were treated with 0, 5, 10, 20, 40, 80, or 160 μM CaA for 12, 24, or 48 h. As shown in Figure 1A, cell viability increased after treatment with 10 and 20 μM CaA; however, there were marked decreases at 40, 80, and 160 μM CaA. Cell viability reached a peak at 10 μM CaA

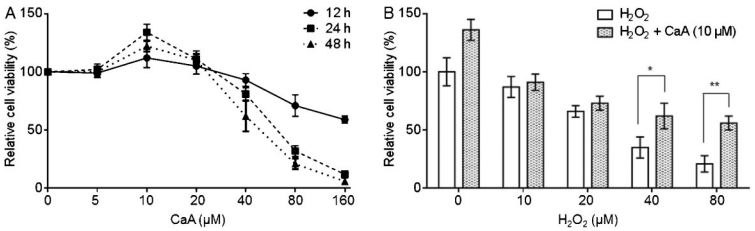

Figure 1. Caffeic acid (CaA) attenuated the H_2O_2-induced inhibition of L-02 cell viability. *A*, L-02 cells were treated with 0, 5, 10, 20, 40, 80, or 160 μM CaA for 12, 24, or 48 h. *B*, After pretreatment of L-02 cells with 0 or 10 μM CaA for 24 h, they were exposed to 0, 10, 20, 40, or 80 μM H_2O_2 for 24 h. Cell viability was measured by cell counting and comparison with control cells treated with culture medium only. *P<0.05 and **P<0.01 compared with L-02 cells treated only with H_2O_2 (Student's *t*-test).

and then declined with increasing concentrations, so 10 μM was chosen for further investigation. We next exposed L-02 cells to H_2O_2, which induces oxidative stress and generates DSBs, and further evaluated the antioxidant effects induced by a low concentration of CaA. As shown in Figure 1B, H_2O_2 decreased cell viability in a dose-dependent manner, but CaA attenuated the H_2O_2-mediated inhibition of cell viability, suggesting that hormesis induced by a low concentration of CaA attenuated the decrease in L-02 cell viability induced by H_2O_2.

CaA decreased the H_2O_2-induced DSBs in L-02 cells

Oxidative DNA damage is the leading cause of decreased cell viability (13,14). We exposed L-02 cells to 0, 10, 20, 40, or 80 μM H_2O_2 for 6 h and found dose-dependent increases in the expression of γ-H2AX, which is a biomarker of DSBs (Figure 2A and B). We then evaluated the ability of CaA to protect against the DNA damage that resulted from H_2O_2 treatment. After pretreating L-02 cells with 0 or 10 μM CaA for 24 h, they were exposed to 40 or 80 μM of H_2O_2 for 6 h. As shown in Figure 2C and D, CaA attenuated the H_2O_2-induced increase in γ-H2AX expression. These results indicate that a low concentration of CaA decreased H_2O_2-induced DSBs in L-02 cells.

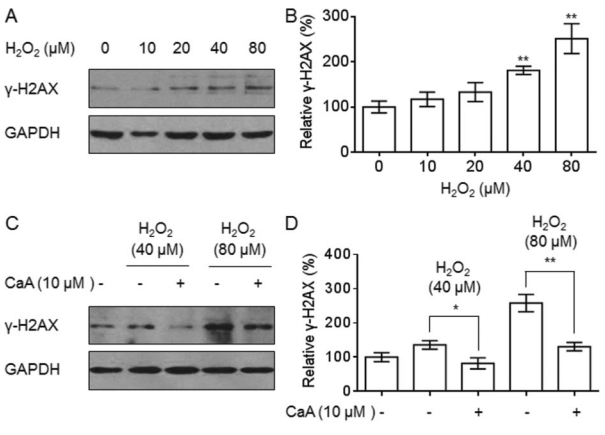

Figure 2. Caffeic acid (CaA) decreased H_2O_2-induced double-strand breads (DSBs) in L-02 cells. *A*, *B*, L-02 cells were exposed to 0, 10, 20, 40, or 80 μM H_2O_2 for 6 h. *A*, Western blot analysis and *B*, relative γ-H2AX protein levels. **P<0.01 compared with control cells. *C*, *D*, L-02 cells were pretreated with 0 or 10 μM CaA for 24 h and then exposed to 40 (*lanes 2* and *3*) or 80 (*lanes 4* and *5*) μM H_2O_2 for 6 h. *C*, Western blot analysis and *D*, relative γ-H2AX protein levels. *P<0.05 and **P<0.01 compared with L-02 cells treated with H_2O_2 alone (Student's *t*-test). Bands were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

CaA decreased ROS levels in L-02 cells

ROS have been implicated in a number of processes including cell proliferation, DNA damage, and apoptosis (9,11,14). At low levels, ROS modulate gene expression by acting as second messengers, but at high levels they cause oxidative injury leading to cell death (13,15). We hypothesized that the CaA-induced improvement of cell viability and protection against DNA damage following H_2O_2 treatment were mediated by the generation of low levels of ROS. To confirm our hypothesis, L-02 cells were exposed to 10 μM CaA for 0, 3, 6, 12, 24, or 48 h. As shown in Figure 3, the ROS levels induced by CaA were 112-136% compared with control cells (100%); however, in cells treated with 40 μM H_2O_2, the relative ROS level was 236%. These results suggest that compared with exposure to H_2O_2, CaA generated relatively lower levels of ROS in L-02 cells.

CaA activated ERK signaling by ROS in L-02 cells

The ERK pathway typically transduces growth factor signals that lead to cell differentiation or proliferation (7); however, the association of ERK with CaA-induced improvement of cell viability and subsequent protection against DNA damage is unclear. We exposed L-02 cells to

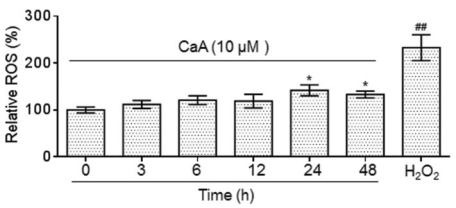

Figure 3. Caffeic acid (CaA) generated relatively lower levels of reactive oxygen species (ROS) in L-02 cells compared to cells treated only with H_2O_2. L-02 cells were treated with 10 μM CaA for 0, 3, 6, 12, 24, or 48 h. Cells exposed to 40 μM H_2O_2 for 24 h served as positive controls. ROS levels were measured with the DCF fluorescence method. *P<0.05 compared to medium control cells; ##P<0.01 compared to medium control cells and to CaA treatment at all times (one-way ANOVA followed by Dunnett's *t*-test).

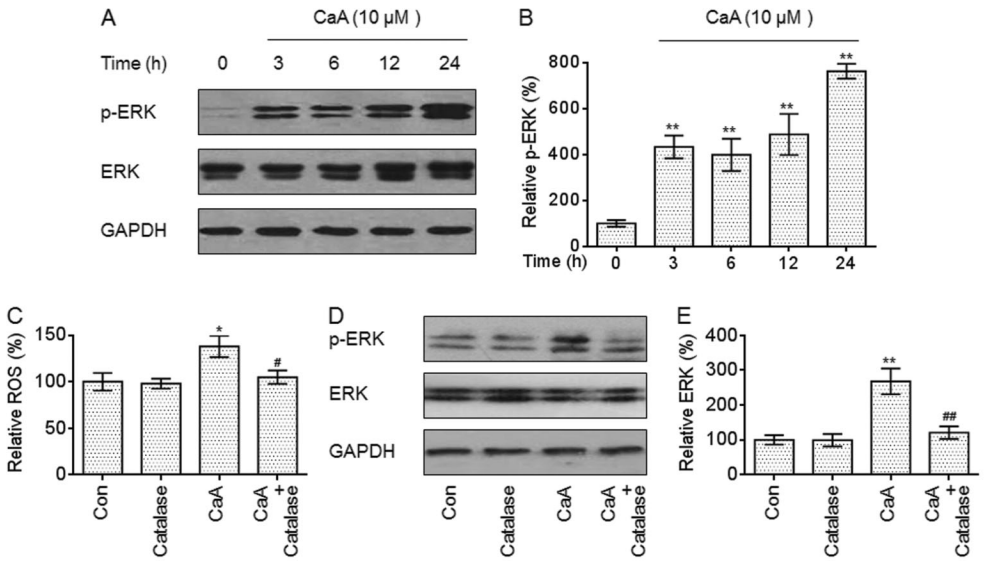

Figure 4. Caffeic acid (CaA) activated extracellular signal-regulated kinase (ERK) signaling by reactive oxygen species (ROS) in L-02 cells. *A*, *B*, L-02 cells were treated with 10 μM CaA for 0, 3, 6, 12, or 24 h. *A*, Western blots and (*B*) relative p-ERK protein levels. *C-E*, L-02 cells were pretreated with 10 nM catalase for 1 h and then exposed to 10 μM CaA for 24 h. *C*, ROS levels were measured with the DCF fluorescence method. The relative ROS ratios were determined by comparison with control cells. *D*, Western blots and *E*, relative p-ERK levels. *P<0.05 and **P<0.01 compared with controls; #P<0.05 and ##P<0.01 compared with cells treated with CaA alone (one-way ANOVA followed by Dunnett's *t*-test).

10 μM CaA for 0, 3, 6, 12, or 24 h, and found that with increased time of CaA exposure, there was enhanced expression of p-ERK, a biomarker for the activation of ERK signaling (Figure 4A and B). Next, we investigated the mechanisms underlying CaA-induced activation of ERK signaling. L-02 cells were pretreated with 10 nM catalase, an H_2O_2 scavenger, for 1 h and then exposed to 10 μM CaA for 24 h. As shown in Figure 4C-E, ROS scavenging by catalase attenuated both the CaA-induced generation of ROS and ERK activation. These results indicate that CaA generated a relatively low level of ROS in L-02 cells, which induced sustained activation of the ERK signal pathway. These results suggest that ERK played a role in the CaA-induced improvement of cell viability and protection against DNA damage that are associated with H_2O_2 treatment.

Inhibition of ERK blocked CaA-mediated reduction of DSBs

We found evidence to support our hypothesis that ERK was involved in the CaA-mediated reduction of DSBs in H_2O_2-treated L-02 cells. Following pretreatment with 0 or 10 μM CaA in the presence or absence of 10 μM U0126 (an ERK inhibitor) for 24 h, cells were exposed to 80 μM H_2O_2 for an additional 6 h. As shown in Figure 5A and B, CaA attenuated the H_2O_2-induced increase in γ-H2AX expression. However, inhibition of ERK by U0126 abolished this phenomenon. RNA interference confirmed the effect of ERK inhibition. In L-02 cells, knockdown of ERK1 and ERK2 by their specific siRNAs blocked the CaA-induced attenuation of γ-H2AX expression in response to H_2O_2 treatment (Figure 5C and D). These results indicate that the ERK signaling pathway was involved in CaA-induced protection against DNA damage by H_2O_2 treatment.

Inhibition of ERK blocked the CaA-induced improvement of cell viability in H_2O_2-treated L-02 cells

Finally, we demonstrated that ERK was involved in the CaA-induced improvement in the viability of cells treated with H_2O_2. L-02 cells were treated as described above for 24 h. As shown in Figure 6, CaA attenuated the H_2O_2-induced decrease in cell viability; however, inhibition of ERK by U0126 or siRNA abolished this effect. These results indicate that the ERK signaling pathway was involved in the CaA-induced improvement of cell viability.

Discussion

Hydroxycinnamic acid derivatives are reported to have anticancer, anti-inflammatory, and antioxidant activities, and CaA is a well known hydroxycinnamic acid (16). Previous studies have demonstrated that daily coffee intake was associated with a reduced incidence of colon and rectal cancer. Michels et al. (17) reported that participants who regularly consumed two or more cups of decaffeinated coffee per day had a 52% lower incidence of rectal cancer than those who never consumed it. Tavani et al. (18) found that compared with coffee nondrinkers, the risk of colon

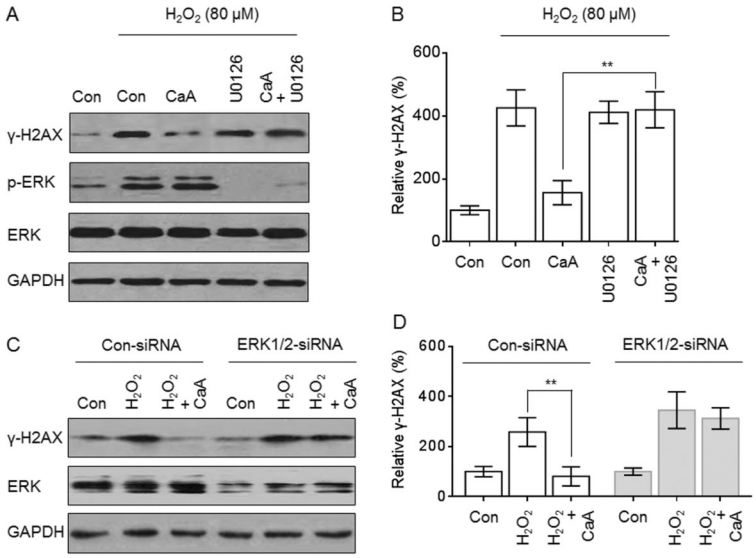

Figure 5. Extracellular signal-regulated kinase (ERK) inhibition blocked the caffeic acid (CaA)-induced reduction in double-strand breads (DSBs) in human L-02 cells. *A*, *B*, After pretreatment with 0 or 10 μM CaA in the presence or absence of U0126 (10 μM) for 24 h, they were exposed to 80 μM H_2O_2 for 24 h. *A*, Western blots and *B*, relative γ-H2AX protein levels. **P<0.01 compared with L-02 cells treated with CaA plus H_2O_2. *C*, *D*, After L-02 cells were transfected with 20 nM ERK1-siRNA plus ERK2-siRNA for 12 h, they were treated with 0 or 10 μM CaA for 24 h, followed by exposure to 80 μM H_2O_2 for an additional 24 h. *C*, Western blots and *D*, relative γ-H2AX protein levels. **P<0.01 compared with L-02 cells treated by H_2O_2 alone (one-way ANOVA followed by Dunnett's *t*-test).

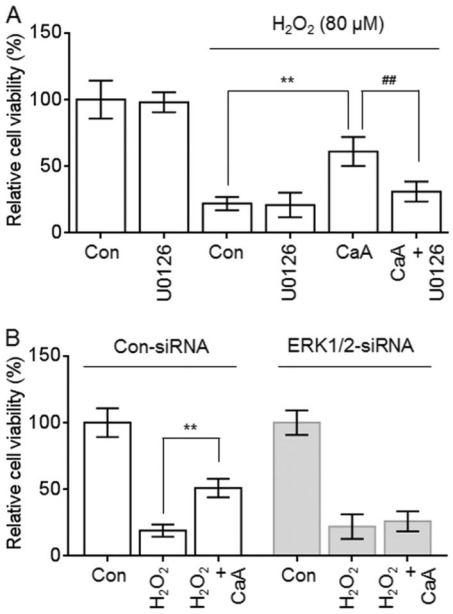

Figure 6. Extracellular signal-regulated kinase (ERK) inhibition blocked the caffeic acid (CaA)-induced improvement of cell viability in H_2O_2-treated L-02 cells. *A*, After pretreatment with 0 or 10 μM CaA in the presence or absence of U0126 (10 μM) for 24 h, they were exposed to 80 μM H_2O_2 for an additional 24 h. Cell viability was measured by cell counting. Relative cell viability was determined by comparison with control cells. **P<0.01 compared with L-02 cells treated with H_2O_2 alone. ##P<0.01 compared with L-02 cells treated with CaA plus H_2O_2. *B*, After L-02 cells were transfected by 20 nM ERK1 plus ERK2 siRNA for 12 h, they were treated with 0 or 10 μM CaA for 24 h, followed by exposure to 80 μM H_2O_2 for an additional 24 h. Cell viability was measured by cell counting. **P<0.01 compared with L-02 cells treated with H_2O_2 alone (one-way ANOVA followed by Dunnett's *t*-test).

cancer was reduced in drinkers of four or more cups per day. In most coffee drinkers, the daily intake of CaA is 0.5-1 g (approximately 0.5-1 mM), and the absorption ratio of CaA is about 95%. Some of the CaA in food enters the circulation, but most passes into the colon (19), so here we used a relatively low concentration of CaA (10 μM) to assess its ability to protect against DNA damage and maintain cell viability after H_2O_2 treatment.

The present study employed L-02 cells because *i*) hepatocellular carcinoma, the most common liver malignancy, is a global health problem (20); *ii*) CaA protects against liver lesions and carcinogenesis in humans (21,22); and *iii*) the liver is thought to be the most important organ for CaA metabolism. Identification of the molecular mechanisms underlying the CaA-induced effects on cell viability and DNA damage would add to our understanding of the anti-oncogenetic effects of CaA.

Biphasic dose-response relationships have recently received considerable attention (23,24). They are characterized by stimulation of chemical agents at low doses (hormesis) and inhibition at high doses (25). Hormesis, also known as oxidative stress adaptation, is an important mechanism by which cells and organisms respond to and cope with environmental and physiological shifts in oxidative stress levels (25). The accumulated evidence for hormesis of chemical agents derives from three different areas: cell proliferation or viability, DNA base excision repair, and telomerase activity (25-27). Here, we established that low levels of CaA act as a hormesis trigger to improve cell viability and protect against DNA damage caused by H_2O_2 treatment.

ROS, such as superoxide anions, H_2O_2, and hydroxyl radicals, are ubiquitous, highly reactive, diffusible molecules (16,28). It has long been recognized that ROS cause complex and irreversible damage to cellular constituents that impairs cellular homeostasis (15). Oxidative damage

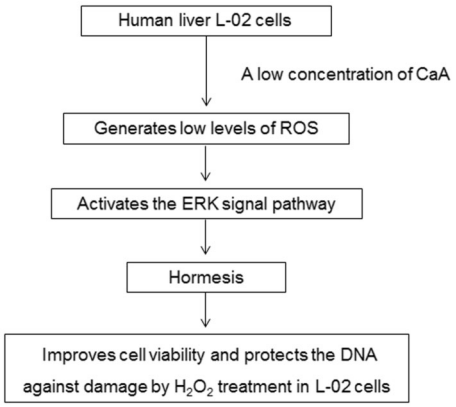

Figure 7. Caffeic acid (CaA)-induced improvement of cell viability and protection against DNA damage: involvement of reactive oxygen species (ROS) and extracellular signal-regulated kinase (ERK) signaling.

is related to the high reactivity of molecular oxygen and its intermediates, which can lead to oxidative modifications of proteins, lipids, and DNA (15). A role for oxidative damage to DNA in carcinogenesis is consistent with accumulating evidence that the rate of genome instability increases with age (4,14). Recent studies suggest that the enhanced ROS generation without cytotoxicity has a cellular protective effect (15,27). On the other hand, marked ROS formation causes oxidative stress and cellular damage (16,29). Here we found that compared with cells exposed to H_2O_2, a low concentration of CaA-generated ROS may play a protective role in L-02 cells. These results are in line with previous findings that sodium arsenite acts as a hormesis trigger at low concentrations and induced enhanced ROS generation without cytotoxicity and had a cellular protective effect (15).

ROS can trigger the activation of redox-sensitive signal transduction and MAPK pathways that regulate cellular mechanisms of cell survival, death, and immunity (9,30,31). MAPKs including ERK, p38 MAPK, and JNK are key components of signaling pathways that control cell differentiation and growth (9,30,31). There is evidence that MAPKs can be phosphorylated and activated in response to oxidant-induced alterations of the redox state (7). After activation, each MAPK phosphorylates a distinct spectrum of substrates including key regulatory enzymes, cytoskeletal proteins, regulators of apoptosis, nuclear receptors, and many transcription factors that bind to specific DNA sequences and induce transcriptional activation and DNA synthesis, with cellular recruitment to the S-phase (9,30,32). CaA regulates LPS-induced oxidative stress through c-Src/ERK signaling pathways in endothelial cells (4). Notably, the ERK signal pathway is involved in the improvement of cell viability induced by a low concentration of sodium arsenite, a hormesis trigger (33). Here we found that a low concentration of CaA induced sustained activation of ERK signaling. Further, we confirmed that the CaA-induced activation of ERK was mediated by ROS generation. Based on these results, we hypothesized that ERK might play a role in CaA-induced improvement of cell viability and protection against DNA damage caused by H_2O_2 treatment. To further understand the role of the ERK pathway, we used U0126 and ERK1/2-siRNA to block ERK activation. Inhibition of ERK blocked the CaA-mediated reduction of DSBs and attenuated the CaA-induced improvement of cell viability associated with H_2O_2 treatment.

A low concentration of CaA increased the viability of human liver L-02 cells, attenuated the H_2O_2-associated reduction of cell viability, and decreased the occurrence of H_2O_2-induced DSBs. Compared with cells exposed to H_2O_2, CaA-treated cells generated lower levels of ROS that induced ERK signaling pathway. Inhibition of ERK signaling blocked the CaA-induced improvement of cell viability and protection against DNA damage caused by H_2O_2 treatment (Figure 7).

Acknowledgments

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References

1. Kang NJ, Lee KW, Shin BJ, Jung SK, Hwang MK, Bode AM, et al. Caffeic acid, a phenolic phytochemical in coffee, directly inhibits Fyn kinase activity and UVB-induced COX-2 expression. *Carcinogenesis* 2009; 30: 321-330, doi: 10.1093/carcin/bgn282.
2. Karthikesan K, Pari L. Beneficial effect of caffeic Acid on alcohol-induced alterations in lipid peroxidation and antioxidant defense in rats. *Toxicol Mech Methods* 2007; 17: 527-534, doi: 10.1080/15376510701410476.
3. Khan AQ, Khan R, Qamar W, Lateef A, Ali F, Tahir M, et al. Caffeic acid attenuates 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced NF-kappaB and COX-2 expression in mouse skin: abrogation of oxidative stress, inflammatory responses and proinflammatory cytokine production. *Food Chem Toxicol* 2012; 50: 175-183, doi: 10.1016/j.fct.2011.10.043.
4. Kim SR, Jung YR, Kim DH, An HJ, Kim MK, Kim ND, et al. Caffeic acid regulates LPS-induced NF-kappaB activation through NIK/IKK and c-Src/ERK signaling pathways in endothelial cells. *Arch Pharm Res* 2014; 37: 539-547, doi: 10.1007/s12272-013-0211-6.
5. Touaibia M, Jean-Francois J, Doiron J. Caffeic Acid, a versatile pharmacophore: an overview. *Mini Rev Med Chem* 2011; 11: 695-713, doi: 10.2174/138955711796268750.

6. Pari L, Karthikesan K. Protective role of caffeic acid against alcohol-induced biochemical changes in rats. *Fundam Clin Pharmacol* 2007; 21: 355-361, doi: 10.1111/j.1472-8206.2007.00505.x.
7. Ma Y, Jin Z, Huang J, Zhou S, Ye H, Jiang S, et al. IQGAP1 plays an important role in the cell proliferation of multiple myeloma via the MAP kinase (ERK) pathway. *Oncol Rep* 2013; 30: 3032-3038.
8. Li L, Eun JS, Nepal M, Ryu JH, Cho HK, Choi BY, et al. Isopsoralen induces differentiation of prechondrogenic ATDC5 cells via activation of MAP kinases and BMP-2 signaling pathways. *Biomol Ther* 2012; 20: 299-305, doi: 10.4062/biomolther.2012.20.3.299.
9. Sun Y, Tang S, Jin X, Zhang C, Zhao W, Xiao X. Opposite effects of JNK and p38 MAPK signaling pathways on furazolidone-stimulated S phase cell cycle arrest of human hepatoblastoma cell line. *Mutat Res* 2013; 755: 24-29, doi: 10.1016/j.mrgentox.2013.04.015.
10. Yang Y, Li Y, Wang K, Wang Y, Yin W, Li L. P38/NF-kappaB/snail pathway is involved in caffeic acid-induced inhibition of cancer stem cells-like properties and migratory capacity in malignant human keratinocyte. *PLoS One* 2013; 8: e58915, doi: 10.1371/journal.pone.0058915.
11. Hao C, Hao W, Wei X, Xing L, Jiang J, Shang L. The role of MAPK in the biphasic dose-response phenomenon induced by cadmium and mercury in HEK293 cells. *Toxicol In Vitro* 2009; 23: 660-666, doi: 10.1016/j.tiv.2009.03.005.
12. Byun MR, Kim AR, Hwang JH, Kim KM, Hwang ES, Hong JH. FGF2 stimulates osteogenic differentiation through ERK induced TAZ expression. *Bone* 2014; 58: 72-80, doi: 10.1016/j.bone.2013.09.024.
13. Haberzettl P, Hill BG. Oxidized lipids activate autophagy in a JNK-dependent manner by stimulating the endoplasmic reticulum stress response. *Redox Biol* 2013; 1: 56-64, doi: 10.1016/j.redox.2012.10.003.
14. Schumacher B. Transcription-blocking DNA damage in aging: a mechanism for hormesis. *Bioessays* 2009; 31: 1347-1356, doi: 10.1002/bies.200900107.
15. Yang P, He XQ, Peng L, Li AP, Wang XR, Zhou JW, et al. The role of oxidative stress in hormesis induced by sodium arsenite in human embryo lung fibroblast (HELF) cellular proliferation model. *J Toxicol Environ Health A* 2007; 70: 976-983, doi: 10.1080/15287390701290832.
16. Chung KS, Han G, Kim BK, Kim HM, Yang JS, Ahn J, et al. A novel antitumor piperazine alkyl compound causes apoptosis by inducing RhoB expression via ROSmediated cAbl/p38 MAPK signaling. *Cancer Chemother Pharmacol* 2013; 72: 1315-1324, doi: 10.1007/s00280-013-2310-y.
17. Michels KB, Willett WC, Fuchs CS, Giovannucci E. Coffee, tea, and caffeine consumption and incidence of colon and rectal cancer. *J Natl Cancer Inst* 2005; 97: 282-292, doi: 10.1093/jnci/dji039.
18. Tavani A, Pregnolato A, La Vecchia C, Negri E, Talamini R, Franceschi S. Coffee and tea intake and risk of cancers of the colon and rectum: a study of 3,530 cases and 7,057 controls. *Int J Cancer* 1997; 73: 193-197, doi: 10.1002/(SICI)1097-0215(19971009)73:2<193::AID-IJC5>3.0.CO;2-R.
19. Olthof MR, Hollman PC, Katan MB. Chlorogenic acid and caffeic acid are absorbed in humans. *J Nutr* 2001; 131: 66-71.
20. Mun AR, Lee SJ, Kim GB, Kang HS, Kim JS, Kim SJ. Fluoxetine-induced apoptosis in hepatocellular carcinoma cells. *Anticancer Res* 2013; 33: 3691-3697.
21. Liu Y, Flynn TJ, Ferguson MS, Hoagland EM, Yu LL. Effects of dietary phenolics and botanical extracts on hepatotoxicity-related endpoints in human and rat hepatoma cells and statistical models for prediction of hepatotoxicity. *Food Chem Toxicol* 2011; 49: 1820-1827, doi: 10.1016/j.fct.2011.04.034.
22. Guerriero E, Sorice A, Capone F, Costantini S, Palladino P, D'ischia M, et al. Effects of lipoic acid, caffeic acid and a synthesized lipoyl-caffeic conjugate on human hepatoma cell lines. *Molecules* 2011; 16: 6365-6377, doi: 10.3390/molecules16086365.
23. Radak Z, Chung HY, Koltai E, Taylor AW, Goto S. Exercise, oxidative stress and hormesis. *Ageing Res Rev* 2008; 7: 34-42, doi: 10.1016/j.arr.2007.04.004.
24. Wang L, Zou W, Zhong Y, An J, Zhang X, Wu M, et al. The hormesis effect of BDE-47 in HepG2 cells and the potential molecular mechanism. *Toxicol Lett* 2012; 209: 193-201, doi: 10.1016/j.toxlet.2011.12.014.
25. Stebbing AR. Hormesis - the stimulation of growth by low levels of inhibitors. *Sci Total Environ* 1982; 22: 213-234, doi: 10.1016/0048-9697(82)90066-3.
26. Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology* 2005; 6: 71-75, doi: 10.1007/s10522-004-7386-7.
27. Kinoshita A, Wanibuchi H, Morimura K, Wei M, Shen J, Imaoka S, et al. Phenobarbital at low dose exerts hormesis in rat hepatocarcinogenesis by reducing oxidative DNA damage, altering cell proliferation, apoptosis and gene expression. *Carcinogenesis* 2003; 24: 1389-1399, doi: 10.1093/carcin/bgg079.
28. Strengert M, Jennings R, Davanture S, Hayes P, Gabriel G, Knaus UG. Mucosal reactive oxygen species are required for antiviral response: role of Duox in influenza a virus infection. *Antioxid Redox Signal* 2014; 20: 2695-2709, doi: 10.1089/ars.2013.5353.
29. Ramyaa P, Padma VV. Ochratoxin-induced toxicity, oxidative stress and apoptosis ameliorated by quercetin--modulation by Nrf2. *Food Chem Toxicol* 2013; 62: 205-216, doi: 10.1016/j.fct.2013.08.048.
30. Cai DT, Jin H, Xiong QX, Liu WG, Gao ZG, Gu GX, et al. ER stress and ASK1-JNK activation contribute to oridonin-induced apoptosis and growth inhibition in cultured human hepatoblastoma HuH-6 cells. *Mol Cell Biochem* 2013; 379: 161-169, doi: 10.1007/s11010-013-1638-2.
31. Zhang H, Tan S, Wang J, Chen S, Quan J, Xian J, et al. Musashi2 modulates K562 leukemic cell proliferation and apoptosis involving the MAPK pathway. *Exp Cell Res* 2014; 320: 119-127, doi: 10.1016/j.yexcr.2013.09.009.
32. Achiwa Y, Hasegawa K, Udagawa Y. Effect of ursolic acid on MAPK in cyclin D1 signaling and RING-type E3 ligase (SCF E3s) in two endometrial cancer cell lines. *Nutr Cancer* 2013; 65: 1026-1033, doi: 10.1080/01635581.2013.810292.
33. He XQ, Chen R, Yang P, Li AP, Zhou JW, Liu QZ. Biphasic effect of arsenite on cell proliferation and apoptosis is associated with the activation of JNK and ERK1/2 in human embryo lung fibroblast cells. *Toxicol Appl Pharmacol* 2007; 220: 18-24, doi: 10.1016/j.taap.2006.12.021.