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L-carnitine alleviates oxidative stress-related damage via MAPK signaling in human lens epithelial cells exposed to H_2O_2

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Abstract. L-carnitine (LC) is well known for its antioxidative properties. The present study aimed to evaluate the effects of LC on human lens epithelial cells (HLECs) and to analyze its regulatory mechanism in cataractogenesis. HLE B-3 cells were cultured with hydrogen peroxide (H_2O_2) and were pretreated with or without LC. The Cell Counting kit-8 assay was used to determine cell viability. Reactive oxygen species (ROS) assay kit was used to measure the cellular ROS production induced by H_2O_2 and LC. In addition, reverse transcription-quantitative PCR and western blot analysis were performed to detect the expression levels of oxidative damage markers and antioxidant enzymes. Notably, ROS overproduction was observed upon exposure to H_2O_2, whereas LC supplementation markedly decreased ROS levels through activation of the antioxidant enzymes forkhead box O1, peroxiredoxin 4 and catalase. Furthermore, LC suppressed the expression of apoptosis-associated genes (caspase-3) and inflammation-associated genes [interleukin (IL)1, IL6, IL8 and cyclooxygenase-2]. Conversely, LC promoted proliferating cell nuclear antigen, cyclin-dependent kinase (CDK)2 and CDK4 expression, which may increase proliferation of HLECs that were incubated with H_2O_2. In addition, epithelial-mesenchymal transition occurred upon ROS accumulation, whereas the effects of H_2O_2 on AQP1 and vimentin expression were reversed upon LC supplementation. Notably, this study revealed that LC restored the oxidant/antioxidant balance and protected against cell damage through the mitogen-activated protein kinase signaling pathway. In conclusion, LC may serve a protective role in curbing oxidative damage and therefore may be considered a potential therapeutic agent for the treatment of cataracts.

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Introduction

Human lens epithelial cells (HLECs) are vulnerable to oxidative stress, which has deleterious effects on lens transparency and ultimately leads to cataracts. Aberrant reactive oxygen species (ROS) accumulation and scavenging are major contributors to oxidative damage (1-3). Alterations in the cellular microenvironment in response to hydrogen peroxide (H_2O_2) manifest as apoptosis and result in the production of pro-inflammatory mediators in HLECs (4). The risk of oxidative damage to the transparent lens may be compensated by the presence of antioxidant enzymes. Notably, catalase (CAT) and peroxiredoxins (PRDXs) are antioxidant enzymes that act as ROS scavengers and potential antioxidant protectors against cataract development (5,6). Forkhead box (Fox)O1 belongs to the FoxO family of transcription factors and, when activated, serves a protective role in antioxidative responses (7). Therefore, it is important to establish an antioxidative approach for preventing the formation and progression of cataracts.

Epithelial-mesenchymal transition (EMT) may be initiated by oxidative stress and manifests in the loss of epithelial characteristics and the acquisition of mesenchymal properties (8,9). Aquaporins (AQPs) are intrinsic plasma membrane proteins that possess H_2O_2 permeability properties. AQP1 protein expression is confined to the lens epithelium, where it acts as an epithelial marker. AQP1 serves a crucial role in the maintenance of ocular lens homeostasis, and its insufficient function may cause cataracts (10). The relationship between EMT and oxidative stress is an important factor in cataract progression.

It is well known that the lens is an organ that lacks nerves (11); therefore, neurodegeneration is not involved in cataract formation. Acetyl-L-carnitine (ALC) is widely used in neurodegenerative diseases due to its neurobiological

effects (12). Due to the effects of ALC on neurodegeneration, which is not involved in cataract formation, the present study aimed to explore the effects of the antioxidant L-carnitine (LC) on cataract prevention, not ALC. LC is a water-soluble, vitamin-like molecule that is naturally found in meat; since its recognition, LC has garnered much attention. LC is a pivotal agent involved in protecting the cell and DNA against damage induced by oxidative stress (13,14). LC protects the ocular surface; for example, LC protects against hyperosmotic stress in dry eye disease (15). It has previously been reported that perturbation of the carnitine shuttle by increased plasma levels of long-chain acylcarnitines leads to a compromised cellular capacity to prevent ROS generation in age-related macular degeneration (16). Despite these findings, the connection between LC and cataract prevention remains unclear. The present study aimed to explore the effects of LC on H_2O_2-induced oxidative damage in HLECs, and to identify the molecular pathways involved in this protection.

Materials and methods

Cell culture. The HLE B-3 cell line was obtained from American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium/F-12 (HyClone; GE Healthcare Life Sciences) containing 20% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 10 μg/l gentamicin at 37˚C in a humidified atmosphere containing 5% CO_2. The cells were incubated with 0, 100, 150, 200, 250, 300 and 350 μM H_2O_2 alone for 24 h, or were pretreated with LC (Sigma-Aldrich; Merck KGaA) at 0, 10, 100, 300, 500 and 700 μM for 16 h. All treatments were carried out at 37˚C in a cell culture incubator. In addition, cells were pretreated with LC (500 μM), ERK inhibitor (FR180204, 1.25 μM) or p38 inhibitor (PD169316, 1.25 μM), or a combination of LC and FR180204 or LC and PD169316, for 2 h prior to treatment with 250 μM H_2O_2 treatment for 24 h. FR180204 and PD169316 were purchased from Sigma-Aldrich; Merck KGaA.

Cell viability assay. The Cell Counting kit-8 assay was used to detect the effects of different concentrations of H_2O_2 and LC on the viability of HLECs. The optical density (OD) was measured using the CCK-8 method (Dojindo Molecular Technologies, Inc.). Cells were placed in 96-well plates at $2x10^3$ cells/well in 200 μl growth medium and were cultured at 37˚C in a humidified incubator with 5% CO_2. Following treatment with H_2O_2 or LC, 10 μl CCK-8 solution was added to each well. After 2 h at 37˚C, the absorbance was measured at 450 nm. Cell survival rate was calculated according to the following formula: Cell viability (%)=(As-Ab)/(Ac-Ab), where As is the average OD value of the experimental group, Ab is the average OD value of the blank group, and Ac is the average OD value of the control group.

Measurement of cellular ROS production. Cellular ROS production was detected using a Reactive Oxygen Species Assay kit (cat. no. CA1410; Beijing Solarbio Science & Technology Co., Ltd.). The DCFH-DA ROS probe, which permeates the cell membrane with no fluorescence, was used according to the manufacturer's protocol. ROS induces the production of fluorescent DCF through oxidizing DCFH. Subsequently, ROS levels can be determined by detecting the fluorescence of DCF. Images were captured using a fluorescence microscope (DM4000 B LED; Leica Microsystems GmbH).

Total RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from HLECs using the RNAsimple Total RNA Extraction kit [cat. no. dp419; Tiangen Biotech (Beijing) Co., Ltd.] in accordance with the manufacturer's protocol. The extracted RNA was quantified using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.) and stored at -80˚C prior to use. First-strand cDNA was synthesized from 2.0 μg total RNA using a M-MLV Reverse Transcriptase kit (Promega Corporation) according to the manufacturer's protocol, and was then stored at -20˚C before use. qPCR was performed using the StepOnePlus™ Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.), and SYBR Premix Ex Taq™ (Takara Biotechnology Co., Ltd.). The thermocycling conditions were as follows: 95˚C for 3 min, followed by 40 cycles at 95˚C for 12 sec and 62˚C for 40 sec, and a final dissociation stage at 95˚C for 15 sec, 65˚C for 1 min and 95˚C for 15 sec. GAPDH served as an internal control and was used to detect the expression levels of genes in HLECs. Relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ fold change method (17). The primer sequences used for RT-qPCR are listed in Table I.

Western blot analysis. Cells treated with H_2O_2 and LC were homogenized using RIPA lysis buffer with protease and phosphatase inhibitor cocktail (Beyotime Institute of Biotechnology). Protein samples were quantified using the bicinchoninic acid protein assay (Thermo Fisher Scientific, Inc.). Equal quantities of proteins per lane (30-50 μg, according to the thickness of the gel) were separated by 10-12% SDS-PAGE, transferred onto PVDF membranes and were then incubated with 5% blocking reagent (5% skim milk) for 1.5 h at room temperature. Subsequently, the membranes were incubated with the following antibodies overnight at 4˚C: Anti-GAPDH (1:1,000; cat. no. ab181602), anti-PRDX4 (1:1,000; cat. no. ab184167), anti-cleaved caspase-3 (1:1,000; cat. no. ab2302), anti-interleukin (IL)-1β (1:1,000; cat. no. ab45692), anti-vimentin (1:1,000; cat. no. ab92547) and anti-cyclooxygenase-2 (COX2; 1:1,000; cat. no. ab179800) (all from Abcam); anti-proliferating cell nuclear antigen (PCNA; 1:1,000; cat. no. 13110), anti-ERK (1:1,000; cat. no. 4695), anti-phosphorylated (P)-ERK (1:1,000; cat. no. 4370), anti-p38 (1:1,000; cat. no. 8690) and anti-p-p38 (1:1,000; cat. no. 4511) (all from Cell Signaling Technology, Inc.). The membranes were washed three times with phosphate buffered saline-0.1% Tween-20 (Beijing Solarbio Science & Technology Co., Ltd.), and were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:10,000; cat. no. ab6721; Abcam) for 2 h at room temperature. Blots were detected using enhanced chemiluminescence reagents (EMD Millipore) and were exposed to chemiluminescent film (Kodak) or using G:BOX F3 (Syngene). The images were analyzed using ImageJ software (v.1.42q; National Institutes of Health).

Statistical analysis. Data are presented as the mean ± standard error of the mean from three independent experiments. The significance of differences between two groups was evaluated using two-tailed Student's t-test, and one-way ANOVA (significant

Table I. Primer sequences of all genes used in quantitative PCR.

Gene	Forward primer	Reverse primer
H-GAPDH	5'-TGCCCTCAACGACCACTTTG-3'	5'-CTGGTGGTCCAGGGGTCTTA-3'
H-PRDX4	5'-GAAGGAACAGCTGTGATCGA-3'	5'-AGAGCATGCTACCACTTCAGT-3'
H-FOXO1	5'-ATGGCTTGGTGTCTTTCTTTTCT-3'	5'-TGTGGCTGACAAGACTTAACTCAA-3'
H-CAT	5'-TCTGGAGAAGTGCGGAGATT-3'	5'-TCCAATCATCCGTCAAAACA-3'
H-Caspase-3	5'-AGCGAATCAATGGACTCTGGA-3'	5'-GGTTTGCTGCATCGACATCT-3'
H-PCNA	5'-CACTCCACTCTCTTCAACGGT-3'	5'-ATCCTCGATCTTGGGAGCCA-3'
H-COX2	5'-ATAACGTGAAGGGCTGTCCC-3'	5'-ATCATCTAGTCCGGAGCGGG-3'
H-IL1	5'-TCATACCAAGGAGAAGTAATAAGCC-3'	5'-ACCAAAGAAGTACAGCGCCAT-3'
H-IL6	5'-CAAACTTCCTGGAGTTCACC-3'	5'-TGTGTCCAATGGACAGGATG-3'
H-IL8	5'-ACCTCACTGTGCAAATTCAG-3'	5'-TATGACTCTTGCTGCTCAGC-3'
H-AQP1	5'-TAACCCTGCTCGGTCCTTTG-3'	5'-AGTCGTAGATGAGTACAGCCAG-3'
H-CDK2	5'-AAATTCATGGATGCCTCTGC-3'	5'-CAGGGACTCCAAAAGCTCTG-3'
H-CDK4	5'-GAACTGACCGGGAGATCAAG-3'	5'-TCAGATCTCGGTGAACGATG-3'
H-α-SMA	5'-TGACATTTGTGAAACTTCGGGT-3'	5'-TGAAGCAATGGTAGCTGGGT-3'
H-Vimentin	5'-AAATGGCTCGTCACCTTCGT-3'	5'-AGAAATCCTGCTCTCCTCGC-3'

α-SMA, α-smooth muscle actin; AQP1, aquaporin 1; CAT, catalase; CDK, cyclin-dependent kinase; COX2, cyclooxygenase-2; H, human; IL, interleukin; PCNA, proliferating cell nuclear antigen; PRDX4, peroxiredoxin 4.

variation was indicated as α=0.05) followed by Dunnett's multiple comparisons test or Tukey's multiple comparisons test were used to evaluate the significance of differences among multiple groups. GraphPad Prism version 7.0 software (GraphPad Software, Inc.) was used to conduct statistical analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

LC reverses the effects of H_2O_2 on viability of HLE B-3 cells. H_2O_2 induced a significant decrease in HLE B-3 cell viability in a dose-dependent manner (Fig. 1A). Subsequently, 250 μM was chosen as the optimal concentration in the subsequent experiments, because it was approximately equal to the IC_{50} of H_2O_2. Conversely, treatment with LC induced minor alterations in cell viability (Fig. 1B), indicating the minimal cytotoxicity of LC. In addition, LC exerted an ameliorating effect on H_2O_2-induced suppression of cell viability; however, this effect was not dose-dependent (Fig. 1C). Notably, cell viability was reduced to some extent when exposed to 700 μM LC alone; therefore, LC concentrations at 100, 300 and 500 μM were chosen for subsequent experiments.

LC decreases the generation of ROS in HLECs and promotes antioxidant production. To determine the role of LC in ROS-induced oxidative damage, HLE B-3 cells were exposed to H_2O_2 with or without LC pretreatment. This study aimed to determine whether exposure to H_2O_2 and LC could modify ROS generation. A marked increase in DCF-positive cells was observed by fluorescence microscopy in HLE B-3 cells exposed to H_2O_2, as shown in Fig. 2A. DCF fluorescence was markedly reduced by LC pretreatment, thus suggesting that LC partially restrained H_2O_2-induced ROS generation in cells induced by H_2O_2.

As shown in Fig. 2B, compared with in the control group, the mRNA expression levels of FoxO1 exhibited a ~50% reduction in response to H_2O_2 treatment, but were increased following treatment with LC. Similarly, compared with in the H_2O_2 group, the mRNA expression levels of the antioxidant enzymes PRDX4 and CAT were increased upon exposure of HLE B-3 cells to LC (P<0.05). Similar results were obtained by western blotting to detect PRDX4 protein expression (Fig. 2C). These findings indicated that LC may exert protective effects on cells suffering from oxidative damage.

LC inhibits H_2O_2-induced increase of apoptosis-associated and inflammation-associated genes. Cleaved-caspase-3 was detected as a marker of apoptosis; its expression was increased in HLECs exposed to H_2O_2. Conversely, pretreatment with LC partially reversed the increase in cleaved-caspase-3 mRNA and protein expression (Fig. 3A and B; P<0.05). Notably, compared with in the control group, H_2O_2 exposure induced a ~1.6-fold increase in cleaved-caspase-3 protein expression levels, as determined by western blotting (Fig. 3B).

The mRNA expression levels of inflammatory markers COX2, IL1, IL6 and IL8 were increased with H_2O_2 exposure (Fig. 3A), indicating the possible involvement of inflammation during cataract progression. LC reversed the inflammatory reaction induced by H_2O_2 exposure; however, the effects were not dose-dependent. Western blot analysis revealed that the protein expression levels of IL-1β were increased following H_2O_2 treatment, whereas these levels were reduced by LC pretreatment (Fig. 3B). Taken together, these data indicated that LC may have a role in reducing H_2O_2-induced apoptosis via alleviating inflammatory responses.

LC restores proliferation and suppresses ROS-induced EMT in HLECs. The expression levels of EMT-associated genes

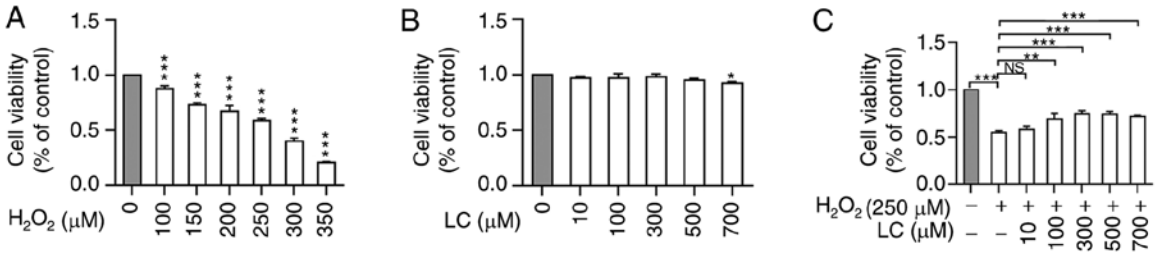

Figure 1. Effects of LC on H_2O_2-induced reductions in HLE B-3 cell viability; cell viability was assessed by Cell Counting kit-8 assay. (A) Cells were cultured with the indicated concentrations of H_2O_2 for 24 h. (B) Cells were cultured with the indicated concentrations of LC for 24 h. Cell viability was slightly inhibited by 700 μM LC. (C) Cells were pretreated with LC at the indicated concentrations for 16 h and were then incubated with 250 μM H_2O_2 for 24 h. The reduction in HLE B-3 cell viability induced by H_2O_2 was restored by LC. Data are expressed as the mean ± standard error of the mean (n=3). *P<0.05; **P<0.01; ***P<0.001; ns, no significant differences (P≥0.05). H_2O_2, hydrogen peroxide; LC, L-carnitine.

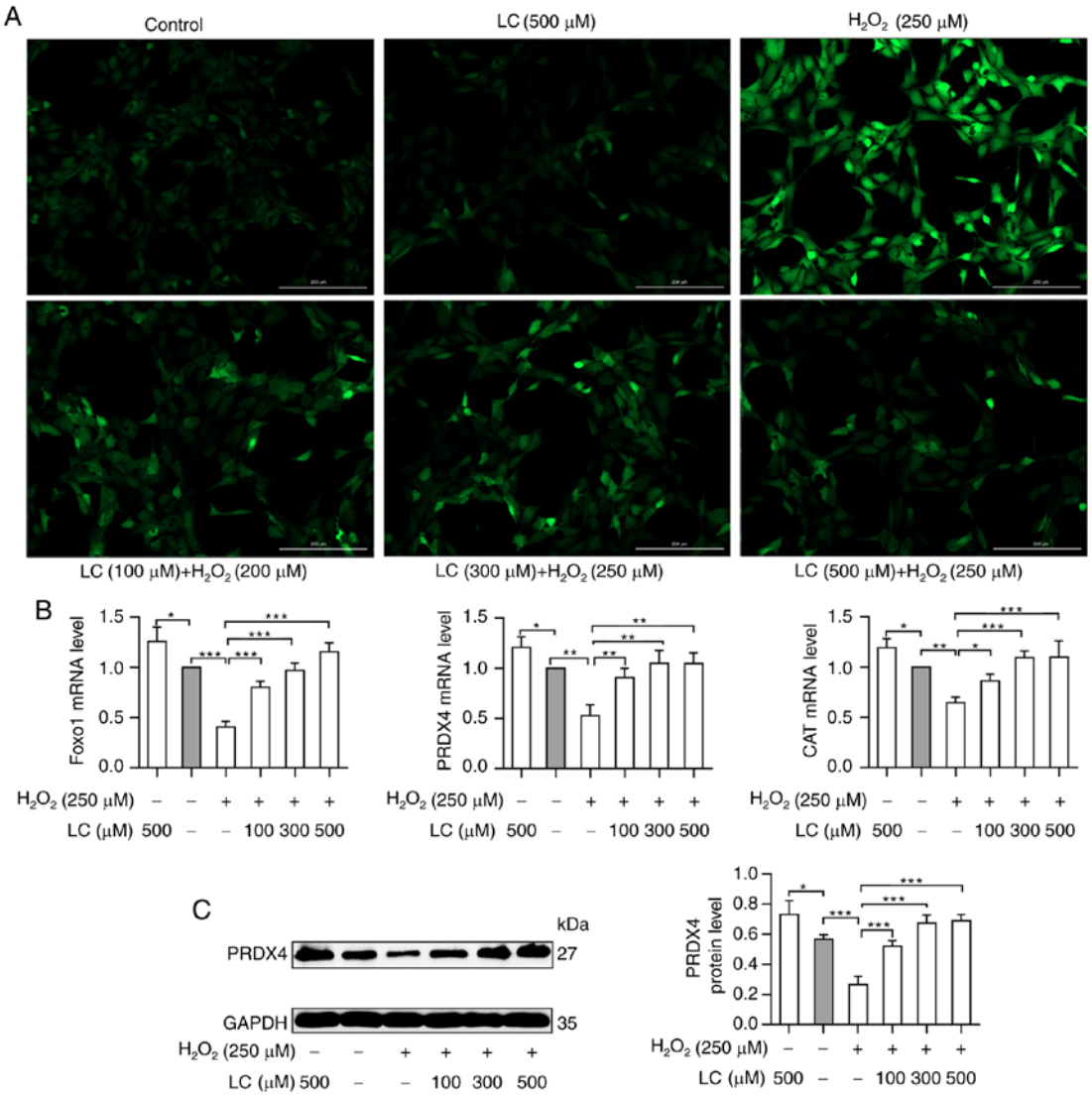

Figure 2. Effects of LC on ROS accumulation and FoxO1, PRDX4 and CAT expression. (A) Increased ROS levels induced by H_2O_2 were reversed by LC treatment in a concentration-dependent manner. Scale bar, 200 μm. (B) Reverse transcription-quantitative PCR analysis of the mRNA expression levels of FoxO1, PRDX4 and CAT. Compared with the H_2O_2 group, FoxO1, PRDX4 and CAT mRNA levels were upregulated by the indicated LC treatment. (C) Western blot analysis of PRDX4. PRDX4 protein levels were significantly elevated in the presence of LC. Gray values were calculated for semi-quantification. Data are expressed as the mean ± standard error of the mean (n=3). *P<0.05, **P<0.01, ***P<0.001. CAT, catalase; FoxO1, forkhead box O1; H_2O_2, hydrogen peroxide; LC, L-carnitine; PRDX4, peroxiredoxin 4; ROS, reactive oxygen species.

were detected in HLE B-3 cells exposed to H_2O_2. The expression levels of AQP1, an epithelial marker, were reduced by H_2O_2. Conversely, the expression levels of the mesenchymal markers vimentin and α-smooth muscle actin (α-SMA) were increased in the H_2O_2 group (P<0.05). Conversely, LC pretreatment significantly reversed the expression patterns

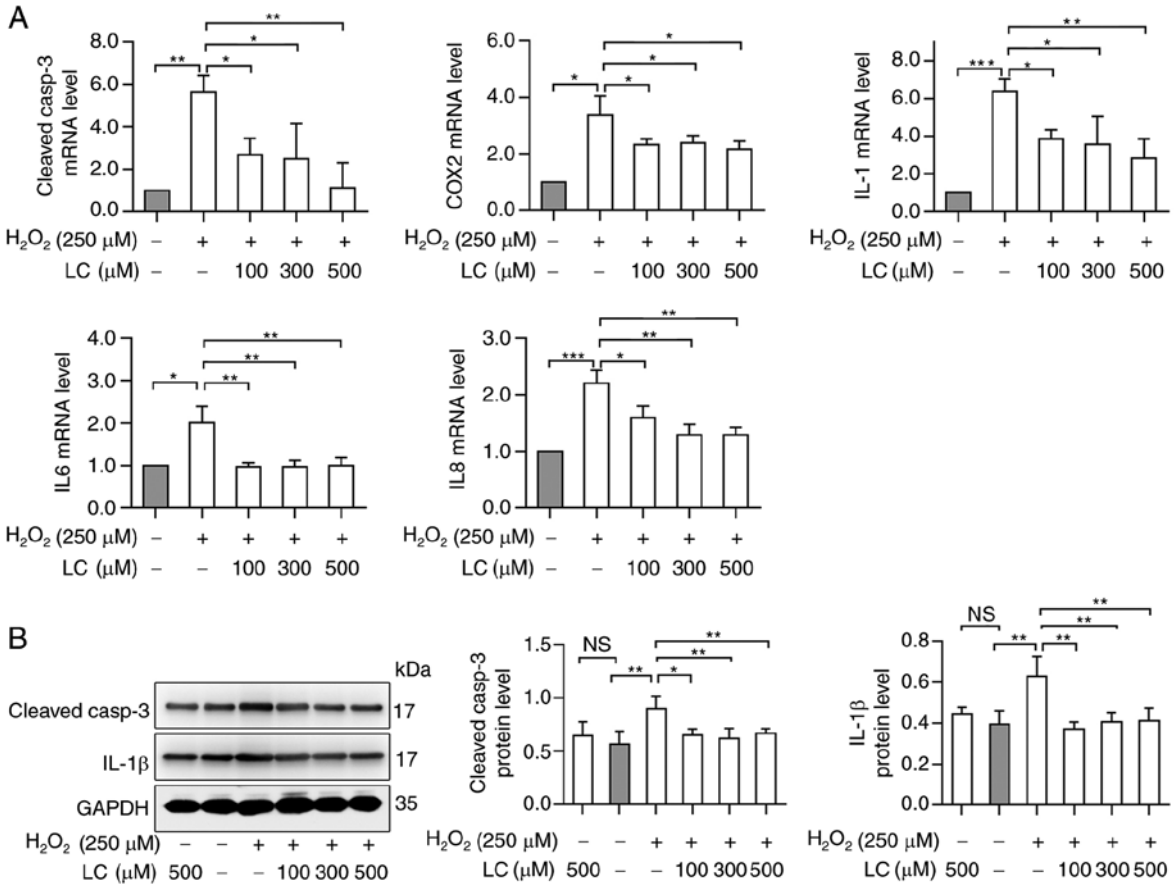

Figure 3. LC inhibits H_2O_2-induced inflammation and apoptosis. (A) Reverse transcription-quantitative PCR analysis revealed that caspase-3, COX2, IL1, IL6 and IL8 levels were reduced by the indicated LC treatment compared with in the H_2O_2 group. (B) Western blot analysis demonstrated that cleaved-caspase-3 and IL-1β levels were reduced by the indicated LC treatment. Gray values were calculated for semi-quantification. Data are expressed as the mean ± standard error of the mean (n=3). *P<0.05, **P<0.01, ***P<0.001; ns, no significant differences (P≥0.05). COX2, cyclooxygenase-2; H_2O_2, hydrogen peroxide; IL, interleukin; LC, L-carnitine.

of the aforementioned genes at the mRNA level (P<0.05; Fig. 4A). Western blot analysis further verified the effects of H_2O_2 and LC on the protein expression levels of vimentin, thus indicating that LC inhibited ROS-induced EMT (Fig. 4B).

Subsequently, the modulatory effects of LC on proliferative markers were analyzed. PCNA expression was decreased to ~70% of the level in the control group in response to H_2O_2 exposure (P<0.05). LC pretreatment increased PCNA expression at the mRNA and protein levels compared with in the H_2O_2 group (Fig. 5A and B). CDK2 and CDK4 mRNA expression was reduced upon H_2O_2 exposure, whereas LC restored their expression (Fig. 5A; P<0.05).

LC regulates oxidative damage through the mitogen-activated protein kinase (MAPK) pathway. Intracellular ROS activates p38 MAPK, an oxidative sensor that belongs to the MAPK family (18); therefore, to decipher the potential cellular mechanism underlying the effects of LC on oxidative damage, this study evaluated whether the MAPK signaling pathway was involved. As determined by western blot analysis (Fig. 5C), p-ERK1, p-ERK2 and p-p38 were significantly enhanced by H_2O_2; however, they were significantly reduced by LC in a dose-dependent manner (P<0.05), thus suggesting the involvement of the MAPK pathway in LC-modified oxidative stress.

To further evaluate the role of the MAPK signaling pathway in mediating the protective effects of LC on H_2O_2-induced cell inhibition and inflammation, cell viability and COX-2 expression was assessed in cells exposed to H_2O_2 and LC in the presence of an ERK inhibitor (FR180204) or p38 inhibitor (PD169316). As shown in Fig. 5D, H_2O_2 markedly enhanced COX-2 expression, whereas LC reversed COX-2 expression. Furthermore, pretreatment with FR180204 or PD169316 abolished H_2O_2-induced COX-2 expression. In addition, HLE B-3 cells exposed to H_2O_2 and LC combined with FR180204 or PD169316 exhibited considerably increased cell viability (Fig. 5E). These results indicated that LC may exert beneficial effects against oxidative damage via MAPK signaling.

Discussion

Oxidative stress is a risk factor for cataracts caused by the overproduction of ROS. H_2O_2 is a main type of ROS that leads to oxidative damage in HLECs. Antioxidants that scavenge excess ROS serve as a defense against cell damage (19). In this study, it was demonstrated that LC exhibited minimal cytotoxicity and reversed H_2O_2-induced ROS production.

Exposure of HLE B-3 cells to H_2O_2 triggered oxidative damage, which was reflected in the destructed antioxidant defense mechanism. Antioxidant substances, including FoxO1, PRDX4 and CAT, are involved in ROS scavenging and serve as potential protectors. H_2O_2-induced oxidative damage is

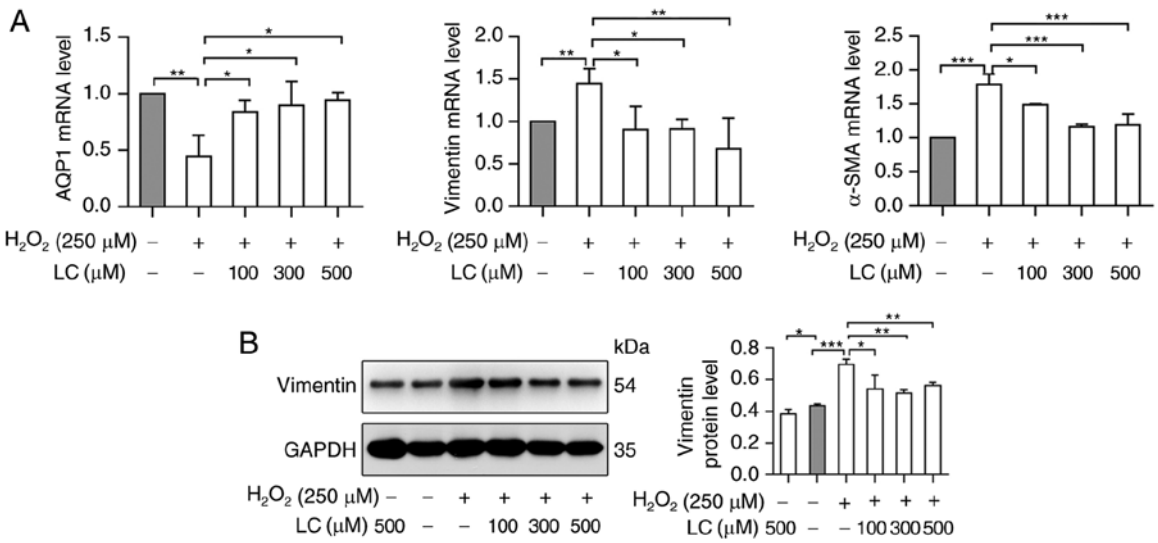

Figure 4. Effects of LC on EMT induced by oxidative stress. (A) Reverse transcription-quantitative PCR analysis revealed that AQP1 expression was increased, whereas vimentin and α-SMA expression was decreased by LC pretreatment compared with in the H_2O_2 group. (B) Western blot analysis confirmed that vimentin expression was decreased by LC; however, the response was not dose-dependent. Data were expressed as the mean ± standard error of the mean (n=3). *P<0.05, **P<0.01, ***P<0.001. α-SMA, α-smooth muscle actin; AQP1, aquaporin 1; H_2O_2, hydrogen peroxide; LC, L-carnitine.

associated with decreased FoxO1, PRDX4 and CAT activities (20,21), indicating the probable mechanisms underlying cataract formation. The present study focused on the oxidative damage caused by ROS imbalance; therefore, FoxO1, PRDX4 and CAT were detected as antioxidative substances.

FoxO1 is highly expressed as a downstream antioxidant when activated (22). The present study revealed that FoxO1 is highly expressed in HLECs treated with LC, suggesting that LC possesses antioxidative potential. The present results indicated that LC may exert beneficial effects on ROS scavenging by increasing the expression levels of the antioxidative enzymes CAT and PRDX4. This finding is consistent with previous findings, which suggested that LC may protect retinal pigment epithelial cells from H_2O_2-induced oxidative damage by increasing antioxidant and antioxidant enzyme activity (23). This study hypothesized that LC may act as a potential antioxidant protector against cataract formation. Our future study aims to further explore alterations in transcriptional regulation and the potential underlying mechanism. In addition, further studies are required to determine the optimal therapeutic delivery method of LC to the lens. Notably, widely used topical inserts and colloidal drug delivery systems (24), such as nanowafers (25), may represent possible pharmacological vehicles to enhance therapeutic efficacy.

PCNA is an auxiliary protein that facilitates cell cycle progression. CDK2 and CDK4 are well-known stimulators and promoters of the cell cycle from the G_0/G_1 into S phase (26,27). In this study, PCNA, CDK2 and CDK4 expression were enhanced by LC in the presence of H_2O_2, demonstrating the role of LC in protecting HLECs against oxidative damage. Given its antioxidant properties, LC may promote the cell cycle and thereby increase cell proliferation.

Exposure to H_2O_2 may promote EMT in the transparent lens. In this study, marked decreases in the expression of the epithelial marker AQP1, together with an increase in mesenchymal markers (vimentin and α-SMA), were observed in HLECs exposed to H_2O_2. Prevention of EMT was demonstrated by elevated AQP1 expression, and attenuated vimentin and α-SMA expression in the presence of LC. This result is consistent with a previous study suggesting that LC prevents the expression of EMT-associated biomarkers in renal fibrosis (28). It was hypothesized that LC may become activated in response to ROS production and scavenging; however, the exact mechanisms require further analysis.

Oxidative stress is closely associated with inflammatory processes, which are important for the initiation and progression of cataracts (29,30). The expression of proinflammatory cytokines, including IL-1β, IL6 and IL8, was reduced by LC pretreatment. COX2 is a major oxygenase, and its expression increases along with oxidative stress-induced inflammation (31). This study further revealed that the production of COX2 was markedly induced by H_2O_2, but significantly rescued by LC. Inflammation triggers LECs to undergo an apoptotic response and subsequently initiate cataract formation (32). Cleaved-caspase-3 expression was decreased and inflammation was inhibited upon LC exposure. In the present study, it was revealed that H_2O_2 may act as a mediator of inflammation and apoptosis in HLECs, whereas LC could significantly attenuate inflammation and reduce apoptosis of HLECs.

The well-known MAPK signaling pathway, which includes p38/MAPK, ERK and JNK, is involved in regulating oxidative damage when cataracts occurs (33,34). The present results indicated that ERK/MAPK and p38/MAPK were significantly activated upon H_2O_2 exposure, whereas LC significantly reduced the phosphorylation of ERK and p38 induced by H_2O_2. Therefore, it was hypothesized that the p38/MAPK and ERK/MAPK pathways are involved in the protective mechanism underlying the effects of LC on oxidative damage in HLECs. It has been reported that MAPK pathway inhibitors can regulate apoptosis and inflammatory responses. The present results revealed that ERK and p38 inhibitors significantly reduced H_2O_2-induced cytotoxicity and

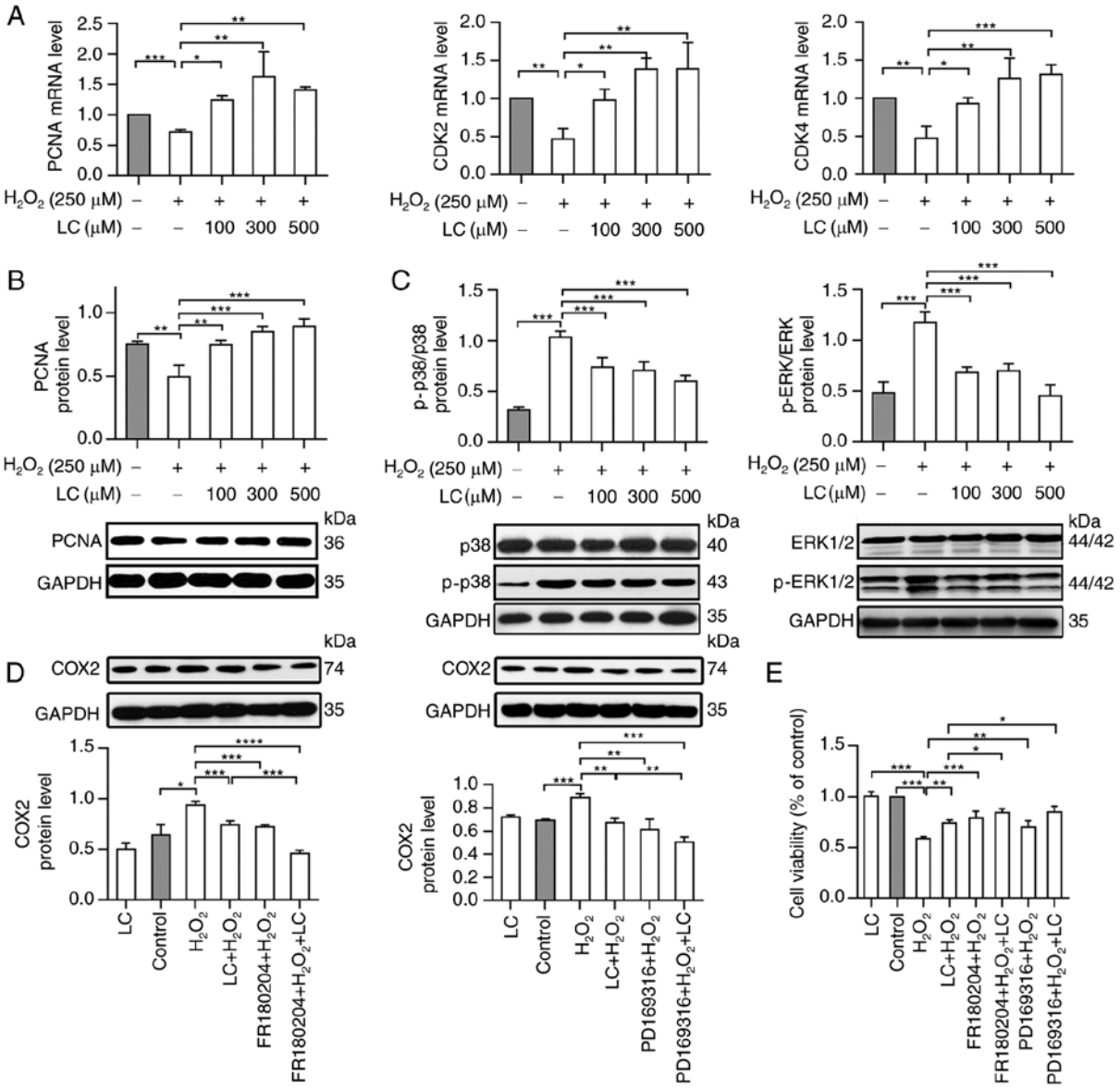

Figure 5. LC restores cell proliferation and regulates cell damage through the MAPK pathway. (A) Relative mRNA expression levels of PCNA, CDK2 and CDK4 were normalized to GAPDH. Compared with in the H_2O_2 group, PCNA, CDK2 and CDK4 mRNA expression was upregulated by LC pretreatment. (B and C) PCNA, ERK1 and ERK2, P-ERK1 and P-ERK2, p38 and p-p38 levels were assessed by western blotting. PCNA was upregulated by the indicated LC treatment, whereas p-p38, P-ERK1 and P-ERK2 were downregulated by LC treatment compared with in the H_2O_2 group. The ERK antibody detected ERK1/ERK2, and the P-ERK antibody detected P-ERK1/P-ERK2. (D and E) Human lens epithelial cells were pretreated with LC (500 μM), ERK inhibitor (FR180204, 1.25 μM) or p38 inhibitor (PD169316, 1.25 μM), or a combination of LC and FR180204 or LC and PD169316, for 2 h prior to treatment with 250 μM H_2O_2 treatment for 24 h. (D) COX2 protein levels were measured using western blotting. Gray values were calculated for quantification. (E) Cell viability was detected by Cell Counting kit-8 assay. Data are expressed as the mean ± standard error of the mean (n=3). *P<0.05, **P<0.01, ***P<0.001. CDK, cyclin-dependent kinase; COX2, cyclooxygenase-2; H_2O_2, hydrogen peroxide; LC, L-carnitine; P-, phosphorylated; PCNA, proliferating cell nuclear antigen.

inhibited the expression of the inflammatory cytokine COX2 induced by exposure to H_2O_2. These findings provide insight into how oxidative modification of LC contributes to cataract prevention.

In conclusion, the protective effects of LC against oxidative stress may be attributed to its ROS-scavenging ability. Oxidative damage in HLECs may be reversed by LC, which prevents the induction of inflammation, apoptosis and EMT through the p38/MAPK and ERK/MAPK pathways. The obtained results suggested that LC may serve an important role in protecting HLECs from peroxidative damage and may be a promising therapeutic modality for the treatment of cataracts.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XY and HL made substantial contributions to the concept and design of the present study. XL, FM, XH and LW performed the experiments. XL analyzed the data and wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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