Caffeic acid methyl ester inhibits LPS-induced inflammatory response through Nrf2 activation and NF-κB inhibition in human umbilical vein endothelial cells

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Abstract. Caffeic acid (CA) derivatives have been reported to exert anti-inflammatory activities in various inflammatory conditions. However, the impact of CA methyl ester (CAME) on the inflammatory response in vascular endothelial cells has not been thoroughly elucidated. In the present study, the aim was to understand how CAME can reduce inflammation in human umbilical vein endothelial cells (HUVECs), which were challenged with lipopolysaccharide (LPS), and elucidate its mechanisms. CAME significantly attenuated LPS-induced TNF-α and IL-1β release. Furthermore, CAME inhibited cyclooxygenase 2 expression and consequent secretion of prostaglandin E_2. CAME also suppressed LPS-stimulated inducible nitric oxide synthase expression. In addition, CAME significantly enhanced the expression of heme oxygenase-1 (HO-1) and nuclear factor erythroid-derived 2-related factor 2 (Nrf2) phosphorylation in the absence or presence of LPS stimulation in HUVECs. CAME also significantly suppressed LPS-induced NF-κB phosphorylation and inhibitor of κB phosphorylation and degradation. In conclusion, the present results provide clear evidence that CAME exerts its anti-inflammatory activities by increasing HO-1/Nrf2-mediated cytoprotection and inhibiting NF-κB-mediated pro-inflammatory pathways in HUVECs.

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Abbreviations: CAME, caffeic acid methyl ester; COX-2, cyclooxygenase 2; iNOS, inducible nitric oxide synthase; HO-1, heme oxygenase 1; Nrf2, nuclear factor erythroid-derived 2-related factor 2; NF-κB, nuclear factor-κB; IκB, inhibitor of κB; IKK, IκB kinase; HUVECs, human umbilical vein endothelial cells; LPS, lipopolysaccharide

Key words: caffeic acid methyl ester, HO-1, Nrf2, NF-κB, IκB, IKK, LPS, HUVECs

Introduction

Endothelial cells produce a variety of inflammatory agents that intensify endothelial dysfunction in inflammatory conditions (1). Inflammation-induced endothelial dysfunction has a crucial impact on the development of multiple vascular diseases, such as hypertension, atherosclerosis and vascular complications associated with diabetes (2,3). Previous research provides substantial evidence to suggest that endothelial cells may have a crucial role in the pathophysiology of sepsis, which is a serious inflammatory response throughout the body to infection triggered by an improper stimulation of the host's immune system by pathogenic elements (4). The pathogenic impact of endothelial activation and dysfunction in sepsis was clearly demonstrated by the unusual increase in cell adhesion molecules, which are involved in various processes related to the adhesion and coagulation of endothelial cells (5,6). This led to a surge in leukocyte migration, coagulation, vascular permeability and inflammation, all of which are typical characteristics of sepsis (7). Studies suggest that cell adhesion molecules and pro-inflammatory agents are enhanced through the activation of the NF-κB signaling pathway (8,9). Thus, mitigating abnormal vascular endothelial activation and the expression of pro-inflammatory mediators could offer substantial therapeutic value in treating inflammation-induced endothelial dysfunction.

Nuclear factor erythroid-derived 2-related factor 2 (Nrf2) is a crucial transcription factor necessary for the cellular response against a wide range of oxidative stressors. A study suggested that Nrf2 demonstrates cytoprotective effects in a sepsis model in mice (10). Caffeic acid (CA) phenethyl ester (CAPE) has been noticed for its role in modulating peripheral immune functions, which includes the expression of heme oxygenase (HO)-1 via the control of Nrf2, a primary transcription factor of HO-1 (11). HO-1, induced by various oxidative

stresses, performs a significant cytoprotective function against oxidative cell damage. The ability of lipopolysaccharide (LPS)-challenged macrophages to defend themselves from the aberrant overproduction of inflammatory mediators such as cyclooxygenase 2 (COX-2) by amplifying HO-1 expression has been reported (12). Furthermore, it has been observed that HO-1 attenuates the overproduction of cytokines such as TNF-α in LPS-challenged RAW264.7 cells (13). In addition, induction of HO-1 has been associated with increased survival rates in fatal endotoxemia (10). An animal study indicated that enhanced expression of HO-1 correlates with significant activation of the Nrf2 pathway (14).

Ester derivatives of CA are found abundantly in numerous natural plants. These include derivatives such as CA methyl ester (CAME), CAPE, CA isopropenyl ester and CA benzyl ester (15-17). Research indicates that they display an array of biological effects, including anti-oxidant, anti-inflammatory, anti-microbial, anti-tumor and anti-acetylcholinesterase activities (17-20). CAPE, for instance, is known to restrain cytokine-induced NF-κB signaling in macrophage cells (21), and plays a critical part in regulating the host's immune response (22). CAPE can also counteract allergic reactions by interfering with MAPK and NF-κB signaling in HMC-1 human mast cells that had been activated (23). CAME has been found to exhibit a variety of pharmacological benefits, such as anti-inflammatory and neuroprotective activities (24). Since CAME demonstrates extensive anti-inflammatory effects and CAPE exhibits anti-allergic characteristics, it is plausible to hypothesize that CAME could also display anti-inflammatory activity in endothelial cells. Therefore, the present study aims to explore the potential anti-inflammatory capabilities of CAME and the underlying mechanism in LPS-stimulated HUVECs, with the goal of identifying a possible therapeutic agent capable of alleviating diverse inflammatory vascular conditions.

Materials and methods

Reagents and cell culture. Escherichia coli serotype 055:B5's bacterial LPS was obtained from Sigma-Aldrich (Merck KGaA). CAME and CA were isolated and identified from the bark of *Lonicera maackii* as described previously (25). To determine whether *Lonicera maackii* is classified as an endangered species, a thorough search was conducted on Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES; https://cites.org/eng). The search confirmed that *Lonicera maackii* is not categorized as an endangered species. Furthermore, *Lonicera maackii* is commonly found in various Asian countries including Korea, Japan and China. To briefly explain the separation and identification process of CAME and CA, the dried bark of *Lonicera maackii*, which were collected in August 2014 from Samaksan (Korea) and identified by Dr Yong-Soo Kwon (College of Pharmacy, Kangwon National University, Chuncheon, Korea). The confirmed sample (no. KNUPH-S-14-01) is currently stored in the medicinal plant laboratory of the College of Pharmacy, Kangwon National University (Chuncheon, Korea). The plant material was dried (2.4 kg), cut into small pieces for use and extracted with 15 l methanol at room temperature. The methanolic extract (230 g) was then partitioned sequentially using hexane, chloroform and butanol. The obtained fractions were subjected to structural determination using NMR and mass spectrometry techniques. In addition, their purity was confirmed to be >95% through high-performance liquid chromatography analysis, which was performed using the Waters e2695 system, and the detector used was the Waters 2489 UV-vis detector (Waters Corp.), measuring at 260 nm. The isolated compounds were identified as CAME and CA by comparing their characteristics with those reported in the compound literature library. *Lonicera maackii* is a shrub of the Caprifoliaceae family, found in Korea, China, Japan and other regions. Its flower buds, leaves and bark are used in traditional medicine for colds and flu. It contains flavonoids, iridoids, caffeoyl quinic acids and phytosterols (25). CAME and CA were dissolved in DMSO and added to the culture media at the required concentrations, with 1 ppm of DMSO in the culture media. Human umbilical vein endothelial cells (HUVECs) were purchased from the American Type Culture Collection (cat. no. CRL-1730) and were cultured on a 2% gelatin-coated plate in M199 medium (Hyclone; Cytiva) (26) supplemented with 20% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 3 ng/ml basic fibroblast growth factor (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.) and 5 U/ml heparin (Sigma-Aldrich; Merck KGaA) (culture medium) in an incubator maintained at ~95% humidity, at a temperature of 37˚C with 5% CO_2. Cells between passages two and six were used in the experiments. The cells were grown on 6-well plates (Corning; Merck KGaA) coated with gelatin and incubated in M199 medium containing 1% fetal bovine serum and 100 U/ml penicillin-streptomycin for 6 h, which is classified as the starvation medium (26). The cells were then incubated with designated concentrations of CAME and CA before being treated with LPS (1 μg/ml). In this study, the micromolar concentration range of CAME was used, as no significant cell toxicity was observed in previous studies (27).

Cytokine and prostaglandin (PG)E_2 assays. HUVECs were incubated with CAME (1-100 μM) for 24 h and then stimulated in the absence or presence of LPS (1 μg/ml) for 24 h. ELISA kits were used to detect TNF-α (cat. no. MTA00B; R&D systems), IL-1β (cat. no. MLB00C; R&D systems) and PGE_2 (cat. no. ADI-900-001; Enzo Life Sciences, Inc.) secreted into the culture media of HUVECs according to the manufacturer's instructions.

Western blot analysis. HUVECs were pretreated with CAME for 3 h before LPS stimulation. HUVECs were rinsed with ice-cold PBS and lysed in PRO-PREP lysis buffer (iNtRON Biotechnology, Inc.). Protein lysates (10 μg per lane) were separated by 10% SDS-PAGE. These proteins were then transferred to Hypond PVDF membranes (Amersham; Cytiva) and blocked for 1 h at room temperature in Tris-buffered saline containing Tween-20 (TBST) with 5% skimmed milk. Specific antibodies against inducible nitric oxide synthase (iNOS; 1:1,000 dilution; cat. no. 610329; BD Pharmingen; BD Biosciences), COX-2 (1:1,000 dilution; cat. no. 12282; Cell Signaling Technology, Inc.), HO-1 (1:1,000 dilution; cat. no. ab13243; abchem), Nrf2 (1:1,000 dilution; cat. no. bs-2013R; Bioss Inc.), phosphorylated

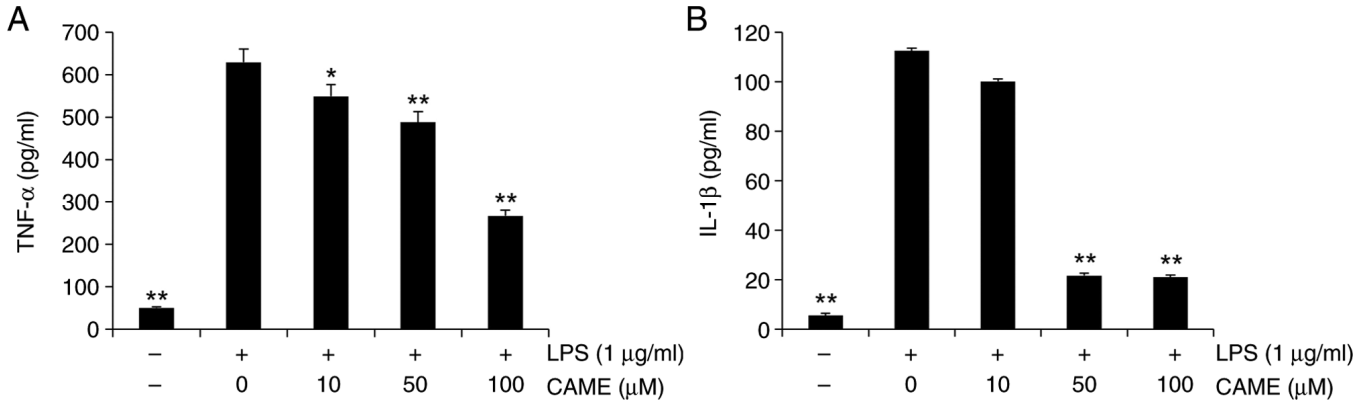

Figure 1. CAME inhibits LPS-induced TNF-α and IL-1β secretion. HUVECs were incubated with 1 μg/ml LPS with or without CAME (10, 50 and 100 μM). (A) TNF-α and (B) IL-1β levels in culture supernatants were determined by ELISA. CAME significantly inhibited the LPS-induced secretion of TNF-α and IL-1β in a dose-dependent manner in HUVECs. The values were presented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01 vs. LPS alone. CAME, caffeic acid methyl ester; LPS, lipopolysaccharide; HUVECs, human umbilical vein endothelial cells.

(p)-Nrf2 (1:1,000 dilution; cat. no. ab76026; abchem), p65 (1:1,000 dilution; cat. no. 8242; Cell Signaling Technology, Inc.), p-p65 (1:1,000 dilution; cat. no. 3033; Cell Signaling Technology, Inc.), inhibitor of kB (IkB; 1:1,000 dilution; cat. no. 9242; Cell Signaling Technology, Inc.), p-IkB (1:1,000 dilution; cat. no. 2589; Cell Signaling Technology, Inc.) and β-actin (1:2,500 dilution; cat. no. A5441; Sigma-Aldrich; Merck KGaA) were diluted in TBST containing 5% milk. After being washed intensely with TBST, the following HRP-conjugated secondary antibodies were added and incubated overnight at room temperature: Anti-mouse IgG (1:1,000 dilution; cat. no. 7076; Cell Signaling Technology, Inc.) or Peroxidase AffiniPure goat anti-rabbit IgG (1:1,000 dilution; cat. no. 111-035-144; Jackson ImmunoResearch, Inc.). The blots were then developed and detected with the use of an enhanced chemiluminescence agent (cat. no. RPN3004; Amersham; Cytiva).

Statistical analysis. Values are expressed as the mean ± SD from three independent experiments. Results were statistically analyzed using SPSS 20.0 (IBM Corp.). To assess the differences between multiple groups, one-way analysis of variance was used, and Dunnett's multiple-comparison test was implemented. P<0.05 was considered to indicate a statistically significant difference.

Results

CAME inhibits TNF-α and IL-1β secretion in LPS-challenged HUVECs. Research studies have reported that inflammatory cytokines such as TNF-α and IL-1β have a vital influence on the progression of inflammation (28,29). Hence, the impact of CAME on the extracellular secretion of TNF-α and IL-1β was examined in LPS-challenged HUVECs. The cells were treated with CAME for 24 h prior to LPS treatment (1 μg/ml). LPS increased the secretion of TNF-α and IL-1β in HUVECs and CAME significantly suppressed the extracellular release of TNF-α and IL-1β in LPS-stimulated HUVECs in a concentration-dependent manner (Fig. 1). Of note, there was no evident cytotoxicity of CAME within the concentration ranges used (data not shown).

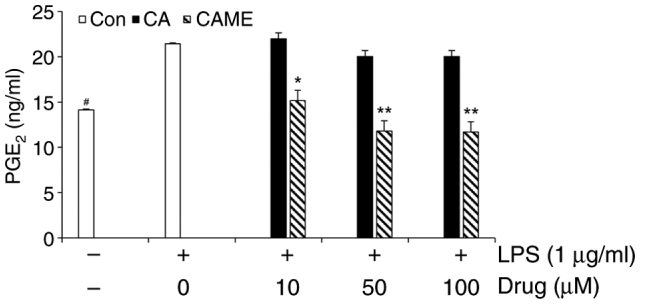

Figure 2. CAME inhibits LPS-induced PGE_2 secretion. HUVECs were incubated with 1 μg/ml LPS with or without of CAME (10, 50 and 100 μM). PGE_2 secretion in culture supernatants was measured by ELISA. CAME significantly inhibited the LPS-induced production of PGE_2 secretion, whereas CA showed no significant inhibition at the same concentration range as that for CAME. Values were presented as the mean ± SD of three independent experiments. *P<0.05, $^{\#}$P<0.05 and **P<0.01 vs. LPS alone. CAME, caffeic acid methyl ester; CA, caffeic acid; con, control; LPS, lipopolysaccharide; PGE, prostaglandin E.

CAME inhibits LPS-induced PGE_2 release and expression of COX-2 and iNOS. Given the previous report that increased levels of COX-2 are associated with inflammatory response leading to the production of pro-inflammatory mediator PGE_2 (22), PGE_2 secretion and COX-2 expression were examined in LPS-challenged HUVECs. LPS treatment resulted in increased secretion of PGE_2 (Fig. 2). To compare the potency of CAME, CA was utilized as a reference compound. Of note, at the concentrations used in the present study, CA exerted no noticeable attenuation on the LPS-induced PGE_2 secretion, whereas CAME showed a significant inhibition of LPS-induced PGE_2 production (Fig. 2), suggesting that CAME may be more potent than CA. Furthermore, the expression level of COX-2 was examined. In accordance with PGE_2 secretion, CAME significantly suppressed LPS-induced COX-2 expression in HUVECs (Fig. 3A). Quantitative analysis of COX-2 expression revealed considerable inhibition of COX-2 expression in a concentration-dependent manner (Fig. 3B). In addition, effects on iNOS, a pro-inflammatory protein related to endothelial inflammation, were examined. CAME significantly inhibited LPS-induced iNOS expression depending on the concentration

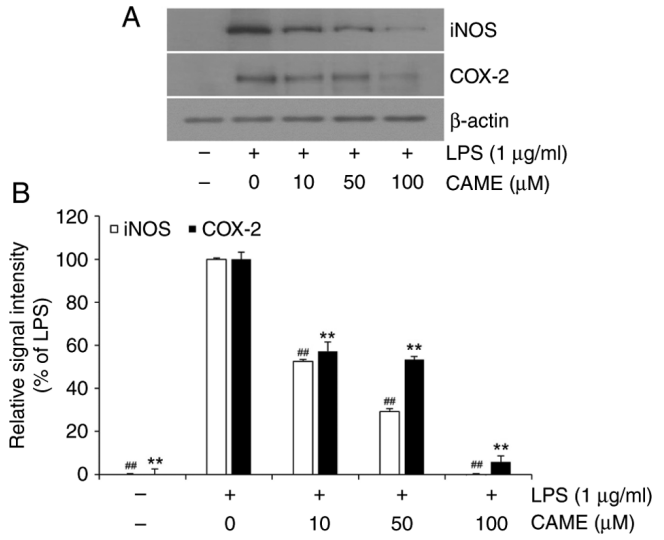

Figure 3. CAME inhibits LPS-induced COX-2 and iNOS expression. Human umbilical vein endothelial cells were incubated with CAME (10, 50 and 100 μM) for 24 h and then challenged with 1 μg/ml LPS for 24 h. COX-2 and iNOS expression was determined by western blotting. CAME significantly inhibited the LPS-induced expression of COX-2 and iNOS in a dose-dependent manner. (A) Representative immunoblots of COX-2 and iNOS. (B) Quantitative analyses of immunoblots of COX-2 and iNOS. The data were presented as the mean ± SD of three independent experiments. **P<0.01 vs. LPS alone for COX-2; $^{\#\#}$P<0.01 vs. LPS alone for iNOS. CAME, caffeic acid methyl ester; LPS, lipopolysaccharide; COX-2, cyclooxygenase 2; iNOS, inducible nitric oxide synthase.

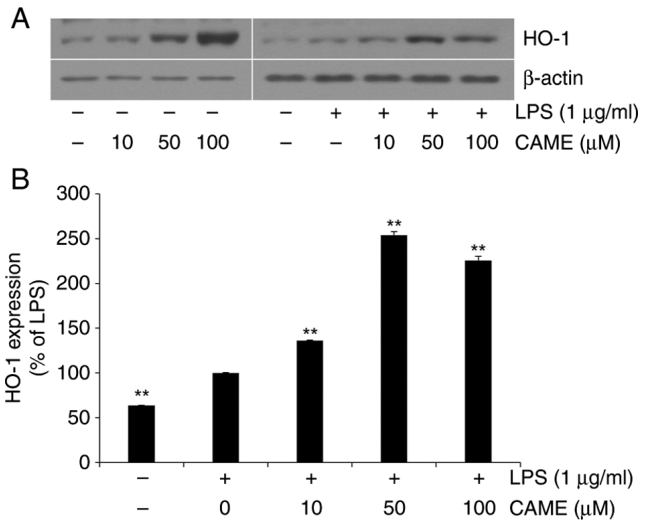

Figure 4. CAME increases the expression of HO-1. Human umbilical vein endothelial cells were treated with CAME (1, 10, 50 and 100 μM) for 3 h and then incubated in the absence or presence of 1 μg/ml LPS for 24 h. CAME significantly increased the expression of HO-1 irrespective of the presence of LPS. (A) Representative image of HO-1 expression and (B) quantitative analysis of immunoblots. β-Actin was employed as a control. The data were presented as mean ± SD of three independent experiments. **P<0.01 vs. LPS alone. CAME, caffeic acid methyl ester; LPS, lipopolysaccharide; HO-1, heme oxygenase 1.

of the compound in LPS-challenged HUVECs (Fig. 3), suggesting that CAME suppresses endothelial inflammation through inhibition of the expression of pro-inflammatory proteins such as COX-2 and iNOS in HUVECs.

CAME activates the HO-1/Nrf2 pathway in LPS-challenged HUVECs. HO-1, which is expressed through the transcription of Nrf2, has been reported to exert cytoprotective effects against a wide range of cellular stresses (30,31). Thus, in the present study, the effect of CAME on the expression of HO-1 was determined. In the absence of LPS challenge, CAME produced an increase in the expression of HO-1 depending on the concentration of the compound in HUVECs (Fig. 4). CAME resulted in increased expression of HO-1 in the presence of LPS challenge, suggesting that CAME exerts cytoprotective effects irrespective of the absence or presence of cellular stresses. Furthermore, the phosphorylation level of Nrf2, the transcription factor of HO-1, was also examined. In accordance with the level of HO-1, Nrf2 phosphorylation was increased with CAME in the absence or presence of LPS treatment (Fig. 5). These results strongly indicate that CAME may exert its cytoprotective activity through activation of the HO-1/Nrf2 pathway.

CAME inhibits NF-κB phosphorylation, and IκB phosphorylation and degradation. NF-κB is a pivotal transcription factor of pro-inflammatory genes in various inflammatory conditions (29). In the present study, the impact of CAME on LPS-induced activation of NF-κB was analyzed in LPS-challenged HUVECs. LPS treatment produced significantly increased phosphorylation of NF-κB

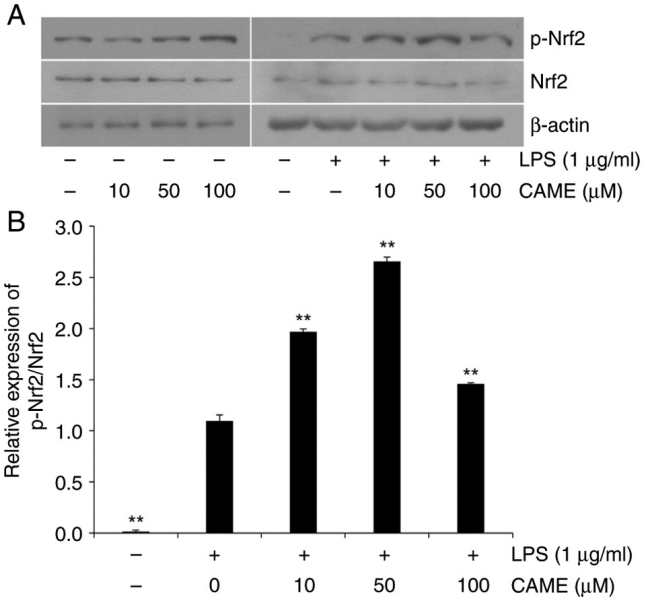

Figure 5. CAME increases the phosphorylation of Nrf2. Human umbilical vein endothelial cells were treated with CAME (1, 10, 50 and 100 μM) for 3 h and then incubated in the absence or presence of 1 μg/ml LPS for 24 h. CAME significantly increased the phosphorylation level of Nrf2 in the absence or presence of LPS. (A) Representative image of Nrf2 phosphorylation and (B) quantitative analysis of immunoblots. β-Actin was used as a control. Values are presented as the mean ± SD of three independent experiments. **P<0.01 vs. LPS alone. CAME, caffeic acid methyl ester; LPS, lipopolysaccharide; p-Nrf2, phosphorylated nuclear factor erythroid-derived 2-related factor 2.

a concentration-dependent manner in HUVECs (Fig. 6). Representative immunoblots showed increased phosphorylation of the p65 subunit and a gradual decrease with CAME

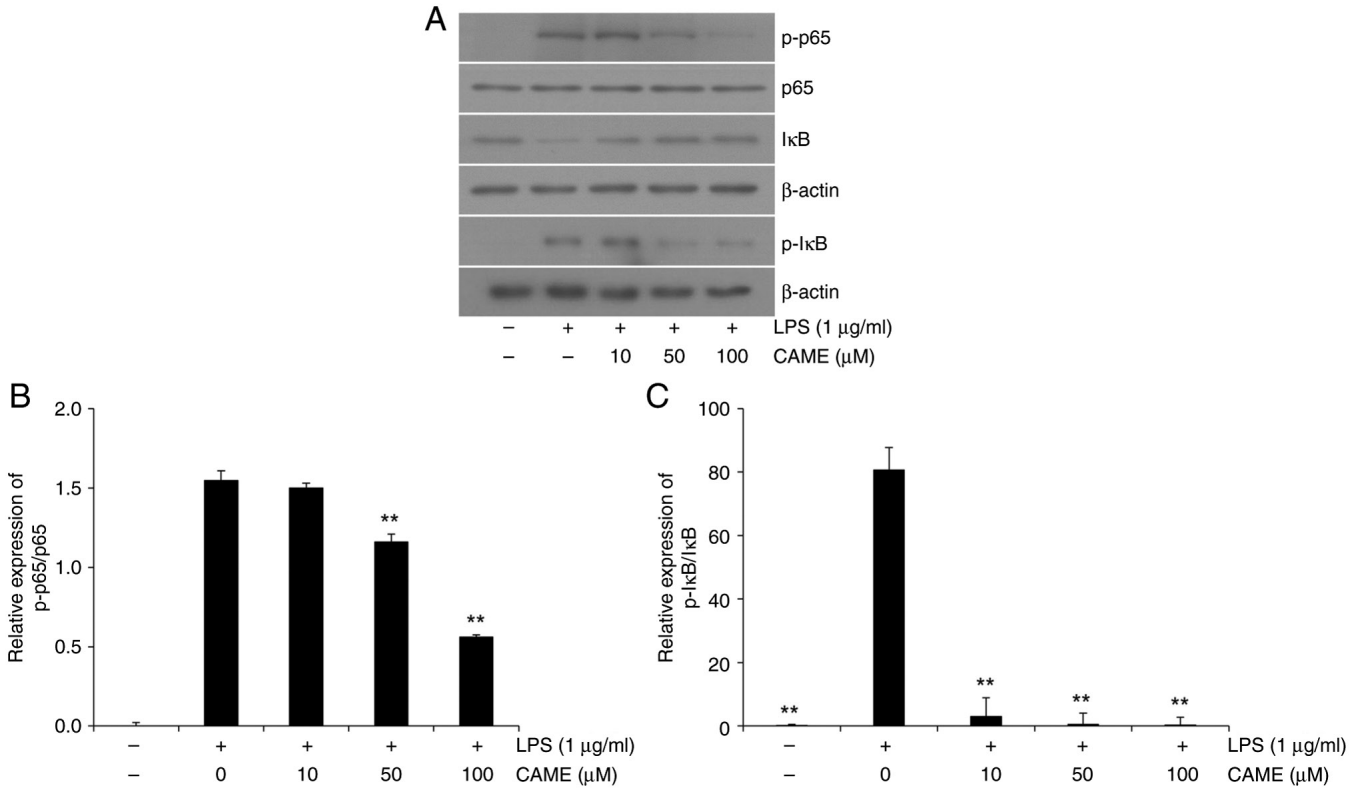

Figure 6. CAME attenuates LPS-induced NF-κB phosphorylation, and IκB phosphorylation and degradation. Human umbilical vein endothelial cells were incubated with CAME (1, 10, 50 and 100 μM) for 3 h and then challenged with 1 μg/ml LPS for 24 h. CAME significantly inhibited the LPS-induced p65 and IκB phosphorylation in a dose-dependent manner. CAME also attenuated LPS-induced degradation of IκB. (A) Western blot images of phosphorylation levels of p65 and IκB, and total level of IκB. Quantitative determination of immunoblots of p-p65 (B) and p-IκB (C). β-Actin was employed as a control. Data were presented as the mean ± SD of three independent experiments. **P<0.01 vs. LPS alone for p-p65. CAME, caffeic acid methyl ester; LPS, lipopolysaccharide; p-IκB, phosphorylated inhibitor of κB.

(Fig. 6A), and quantitative analysis showed a significant decrease of p65 phosphorylation in a concentration-dependent manner (Fig. 6B). LPS-induced phosphorylation of IκB was significantly attenuated by CAME in a concentration-dependent manner (Fig. 6C). As IκB prevents the translocation of the phosphorylated p65 subunit of NF-κB into the nucleus (29), the total level of IκB was also examined. IκB levels were significantly attenuated with the LPS treatment in HUVECs and CAME suppressed IκB degradation in a dose-dependent manner (Fig. 6A and C). Presumably, sustained levels of IκB by CAME sequester NF-κB in the cytosol, preventing the NF-κB-mediated transcription of pro-inflammatory genes. Thus, CAME inhibited LPS-induced NF-κB activation through suppression of IκB degradation.

Discussion

The findings of the present study demonstrated that CAME is effective in reducing inflammatory responses triggered by LPS in vascular endothelial cells. CAME significantly reduced the levels of cytokines secreted, as well as the expression of COX-2 and iNOS in HUVECs challenged with LPS. CAME significantly activated the HO-1/Nrf2 pathway and suppressed the aberrantly activated NF-κB pathway in HUVECs.

CA and their derivatives are known to possess a diverse range of biological properties, such as antitumor, anti-inflammatory, antimicrobial, immunosuppressive and neuroprotective activities (32-34). In a previous study by our group, trihydroxycinnamic acid (THC), a derivative of CA, was shown to exert anti-inflammatory effects in LPS-challenged BV2 microglial cells (35). Our group also reported that THC leads to a reduction of LPS-induced inflammatory reactions in RAW264.7 macrophage cells and enhanced survival rates in mice in an LPS-induced endotoxemia model (9).

Pathologic endothelial activation has been observed in sepsis and numerous cardiovascular conditions, including atherosclerosis, hypertension and hemolysis (36,37). During the inflammatory response, vascular endothelial cells excessively produce a range of cytokines and mediators, thereby exacerbating the inflammatory injury (38). In the present study, the LPS-induced overproduction of cytokines, such as TNF-α and IL-1β, was significantly reduced with CAME treatment. The vascular endothelium acts as a barrier that selectively regulates the transfer of plasma and cells between the bloodstream and adjacent tissues (39). In a previous study by our group, it was observed that LPS-induced vascular leakage in multiple organs, encompassing the spleen, kidney and liver, was substantially curtailed by THC, a CA derivative, in a mouse model of sepsis (9). Furthermore, THC significantly reduced the infiltration of macrophages in the kidneys in an LPS-induced mouse model of sepsis (9). These results suggest that ester derivatives including CAME may also possess protective properties against vascular damage.

Nrf2 has been shown to have an essential role in maintaining cellular homeostasis and suppressing inflammatory conditions through the expression of cytoprotective enzymes and stress-responsive proteins (40,41). In experiments involving mice treated with sulforaphane, activation of Nrf2 and an enhancement of HO-1 were observed (42), while the absence of Nrf2 signaling led to increased vulnerability to inflammation (43). In addition, the upregulation of HO-1 contributed to a decrease in oxidative stress by eliminating free heme and simultaneously increasing the level of anti-inflammatory substances (44). Studies have indicated that the activation of the HO-1/Nrf2 signaling pathway may be facilitated via a variety of signaling pathways (9,10,35). CAPE has been reported to activate the Nrf2 pathway in colonic inflammation (45) and osteoarthritis progression (46). In the present study, treatment with CAME resulted in increased levels of phosphorylated Nrf2, which in turn induced the increased expression of HO-1 in HUVECs in the absence or presence of LPS stimulation. These results imply that the HO-1/Nrf2 signaling pathway may be induced with activators such as CAME, irrespective of the presence of cellular stressors. Furthermore, the HO-1/Nrf2 pathway may also be activated as a defense mechanism against cellular stressors. This suggests that activating the HO-1/Nrf2 pathway may prepare cells for the potential stressor under normal conditions and also provide a defense against damage under stress conditions. Given the cytoprotective role of HO-1, the pharmacological induction of HO-1 expression with CAME may serve as a promising therapeutic approach in combating sepsis and inflammatory vascular conditions. However, further studies CA derivatives activate Nrf2 phosphorylation.

NF-κB is the key transcription factor responsible for inflammatory responses (35). It is widely recognized that NF-κB is implicated in the generation of pro-inflammatory cytokines such as IL-1β and TNF-α, as well as proteins such as COX-2 and iNOS (47,48). LPS has been known to activate the IκB kinase (IKK) complex, composed of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (NEMO/IKKγ). Once activated, IKK phosphorylates the IκB proteins, targeting ubiquitination and subsequent proteasomal degradation. This allows the NF-κB dimers (p50/p65) to translocate to the nucleus and induce the transcription of target genes. Given the fact that inhibition of IKK can prevent the activation and nuclear translocation of NF-κB, IKK inhibitors are potential drugs to suppress the production of cytokines and other inflammatory mediates, ultimately reducing inflammation in tissue damage in various disorders, such as rheumatoid arthritis, inflammatory bowel disease and psoriasis (49,50). Previously, it was reported that CA regulates the NF-κB signaling pathway by acting on the IKK pathway (51). In the present study, CAME significantly suppressed LPS-induced IκB degradation, suggesting a possibility that CAME may directly interfere with the metabolizing action of IKK. Therefore, further studies assessing the involvement of IKK by determining its phosphorylation level and investigating nuclear translocation of NF-κB through a nuclear fractionation assay may offer a more comprehensive understanding of the complete role of the NF-κB pathway.

Of note, the present study had a limitation. CAME, used in the present study, was derived from *Lonicera maackii* and had a purity of >95%. However, it contained certain impurities. There is a chance that these impurities may have influenced the study's anti-inflammatory results. To address this limitation, future studies should compare the effects with CAME commercially purified to 100% to validate the findings of the present study. In addition to CAME, various other compounds, such as 5-caffeoylquinic acid n-butyl ester, methyl 3,4-dicaffeoyl quinate, 3,5-dicaffeoyl quinic acid n-butyl ester, loganin and CA have been isolated from *Lonicera maackii*. While the current study investigated the anti-inflammatory properties of CAME, it does not offer a comprehensive understanding of the biological effects of *Lonicera maackii*. Consequently, in-depth investigations into each individual compound, as well as the whole extract, are crucial for an extensive understanding of the biological properties of *Lonicera maackii*.

In conclusion, the findings of the present study provide clear evidence that CAME effectively suppresses LPS-triggered inflammatory responses by activating the cytoprotective HO-1/Nrf2 pathway and inhibiting pro-inflammatory NF-κB signaling in LPS-challenged HUVECs. CAME can activate HO-1/Nrf2 pathway under normal conditions to preemptively prepare cells for potential stressors and also provide protection against stressors in inflammatory conditions. The results clearly suggest that CAME is a promising therapeutic agent having dual beneficial properties against inflammatory vascular disorders.

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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

JYP and MY conducted the experiments and analyzed the data. ETH, WSP and JHH performed data analysis for the study. YSK isolated and identified CAME and CA from *Lonicera maackii*. HJL performed the statistical analysis of the results. WC conceptualized the study and wrote, reviewed and edited the manuscript. YSK and WC confirm the authenticity of all the raw data. All authors have 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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