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Original Article

Cytotoxic and apoptotic effects of caffeate derivatives on A549 human lung carcinoma cells

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Abstract

Background: Caffeate derivatives have been reported to exhibit antioxidant, anti-inflammatory, and anticancer activities. To reveal the cytotoxic and apoptotic effects of caffeate derivatives, we studied the effects of octyl, phenylpropyl, and decyl caffeates on cell growth and apoptosis in A549 human lung carcinoma cells.
Methods: A549 human lung carcinoma cells were treated with 0–100 μM of caffeate derivatives for 0–48 hours. The cytotoxic and apoptotic effects were evaluated by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay for cell viability, propidium iodide staining method for cell morphology, mitochondrial membrane potential analysis, and Western blot for protein expression.
Results: Octyl, phenylpropyl, and decyl caffeates all significantly decreased the cell viability of A549 cells with 50% inhibitory concentration values of 54.2 ± 10.1 μM, 80.2 ± 1.3 μM, and 74.9 ± 2.1 μM, respectively. Propidium iodide staining revealed that apoptotic bodies appeared when cells were treated with octyl and decyl caffeates. Treatment of A549 cells with octyl and decyl caffeates caused the loss of mitochondria membrane potential. Western blots revealed that octyl and decyl caffeates stimulate an increase in the protein levels of Fas, FasL, and Apaf-1. Moreover, these compounds changed the levels of pro- and antiapoptotic Bcl-2 family members and induced the activation of caspase-12, -9, and -3, which was followed by cleavage of poly (ADP-ribose) polymerase.
Conclusion: These results demonstrate that octyl and decyl caffeates induce cell apoptosis in A549 human lung carcinoma cells.

Keywords: A549 cells; apoptosis; caffeate derivatives; protein expression

1. Introduction

Lung cancer is the most lethal of all cancers worldwide and has a dismal prognosis. It can be divided into two types, small-cell lung cancer and non–small-cell lung cancer. Lung cancer is the most common source of brain metastases in adult patients, which occurs in 30–50% of total lung cancer cases.[1] Non–small-cell lung cancer is the most common type of lung cancer (accounting for 80% of total lung cancer cases)

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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and is divided into three main types, including adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma.[2] Resistance to anticancer agents is a significant problem and is observed frequently in the treatment of patients with small-cell lung cancer and non–small-cell lung cancer.[3]

To date, phytochemicals and their derivatives have played an important role in anticancer drug development. Caffeic acid and some of its derivatives, such as caffeic acid phenethyl ester (CAPE) have been reported to exhibit antioxidant, anti-inflammatory, and anticancer activities.[4] The preincubation of caffeate derivatives (including CAPE, methyl caffeate, ethyl caffeate, butyl caffeate, octyl caffeate, and benzyl caffeate) inhibited nitric oxide (NO) production in lipopolysaccharide-induced RAW 264.7 macrophages,[5] which demonstrates their *in vivo* anti-inflammatory properties. Uwai et al[6] found that the alkyl side-chain of caffeate derivatives inhibited NO production in lipopolysaccharide -induced RAW 264.7 macrophages. Ujibe et al[7] indicated that octyl caffeate induced apoptosis in human leukemia U937 cells through the induction of caspase-3 activity and the fragmentation of nuclei and DNA. Fiuza et al[8] found that propyl caffeate and octyl caffeate have an inhibitory effect on cell growth in human cervix adenocarcinoma cells (HeLa). Nagaoka et al[9] indicated that the oral administration (2 mg/mouse/day) of CAPE, 4-phenylbutyl caffeate, 8-phenyl-7-octenyl caffeate, 2-cyclohexylethyl caffeate, and *n*-octyl caffeate caused a 55%, 43%, 55%, and 35% reduction in the formation of lung metastasis tumor nodules, respectively. Kudugunti et al[10] showed that CAPE induces apoptosis in SK-MEL-28 human melanoma cells through quinone formation, reactive oxygen species formation, intracellular GSH depletion, and induced mitochondria toxicity. Lin et al[11] indicated that caffeic acid induced apoptosis in lung cancer cells through the NF-κB pathway. However, the literature remains unclear regarding the anticancer activity of octyl caffeate, phenylpropyl caffeate, and decyl caffeate in A549 human lung cancer cells.

The objective of this study was to investigate the ability of octyl caffeate, phenylpropyl caffeate, and decyl caffeate to induce apoptosis in A549 human lung carcinoma cells and to examine the chemotherapeutic potential of these derivatives against lung cancer. The effects of caffeate derivatives on intrinsically and extrinsically mediated apoptosis-induction pathways in A549 human lung carcinoma cells were also investigated.

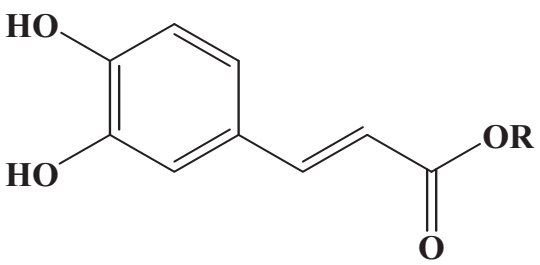
2. Methods

2.1. Materials

Octyl caffeate, phenylpropyl caffeate, and decyl caffeate were provided and synthesized by Professor Yueh-Hsiung Kuo (Tsuzuki Institute for Traditional Medicine, College of Pharmacy, China Medical University, Taichung, Taiwan). Syntheses of caffeate derivatives were obtained from the caffeic acid and thionyl chloride in dry dichloromethane, and ROH and triethylamine were added as a reaction solution. The reaction mixture was partitioned between ethyl acetate and H_2O, and the ethyl acetate layer was washed and dried. The synthetic compound was purified by column chromatography on silica gel and recrystallized from acetone to obtain pure crystals. A Bruker Avance DRX-500 spectrometer (Ettlingen, Germany) was used to record 1H-nuclear magnetic resonance spectra. Electron impact mass spectra were determined on a Finnigan TSQ-46C mass spectrometer (Waltham, CA, USA). Infrared spectra were recorded on a Nicolet Mangna-IR 550 spectrophotomer (Madison, AL, USA). The chemical structures of octyl caffeate, phenylpropyl caffeate, and decyl caffeate are shown in Fig. 1. Sodium bicarbonate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye, and propidium iodide (PI) were purchased from the Sigma Chemical Co. (St Louis, MO, USA). Dimethylsulfoxide was purchased from the Merck Co. (Darmstadt, Germany). RPMI 1640 medium, fetal bovine serum, nonessential amino acids, L-glutamine, sodium pyruvate and the antibiotic mixture (penicillin–streptomycin) were purchased from Invitrogen (Carlsbad, CA, USA). Anti-β-actin, anti-Fas, anti-FasL, anti-AIF (apoptosis-inducing factor), anti-Apaf-1 (apoptotic protease activating factor-1), anti-Fas-associated death-domain-like IL-1β-converting enzyme-inhibitory protein (FLIP), anti-Bcl-2, anti-Bax, anti-Bad, anti-caspase-12, anti-caspase-9, anti-caspase-3, and anti-PARP [poly-(ADP-ribose) polymerase] antibodies were purchased from BioVision (Mountain View, CA, USA). Anti-rabbit or anti-mouse secondary horseradish peroxidase antibodies were purchased from Bethyl Laboratories (Montgomery, TX, USA). Protein molecular mass markers were obtained from Pharmacia Biotech (Saclay, France). Polyvinyldifluoride membrane for Western blotting was obtained from Perkin-Elmer Life Science (Boston, MA, USA). All other chemicals were reagent grade.

2.2. Cell culture

A549 human lung carcinoma cells were obtained from the Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan). A549 cells were grown in a culture medium (90% RPMI 1640, 10% fetal bovine serum, 0.1 mM nonessential amino acid, 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 units/mL penicillin and streptomycin) and incubated at 37°C in a humidified incubator with 5% CO_2.



Octyl caffeate: R = C_8H_{17}

Phenylpropyl caffeate: R = $(CH_2)_3Ph$

Decyl caffeate: R = $C_{10}H_{21}$

Fig. 1. The chemical structures of caffeate derivatives.

2.3. Cell viability

The MTT assay was performed according to the method first described by Mosmann.[12] A549 cells were plated into 96-well microtiter plates at a density of 10^4 cells/well. After 24 hours, culture medium was replaced by 200 μL serial dilutions (0–250 μM) of caffeate derivatives (including octyl caffeate, phenylpropyl caffeate, and decyl caffeate), and the cells were incubated for 24–48 hours. Culture medium was removed and replaced with 90 μL fresh culture medium. A 10-μL aliquot of MTT solution (5 mg/mL) was added to each well reaching a final concentration of 0.5 mg/mL. After 3 hours, unreacted dye was removed, and the insoluble formazan crystals were dissolved in 200 μL dimethylsulfoxide/well and measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 570 nm. The relative cell viability (%) compared to the control (i.e., cells cultured without the caffeate derivatives) was calculated by:

$$A_{570nm}[\text{sample}]/A_{570nm}[\text{control}] \times 100. \quad (1)$$

The 50% inhibitory concentration (IC_{50}) value was calculated as the concentration of the caffeate derivative at which cell growth was inhibited by 50% compared to the untreated controls.

2.4. Nuclear staining with PI

A549 cells were stimulated with 0–50 μM of caffeate derivatives for 48 hours. PI-stained cells were fixed with 80% ethanol for 30 minutes and incubated with 40 μg/mL PI solution for 30 minutes in the dark. The nuclear morphology of the cells was examined using a Motic AE31 inverted microscope with epi-fluorescence microscopy.

2.5. Mitochondria membrane potential assay

Mitochondria membrane potential assays were performed using the JC-1 mitochondria membrane potential assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). A549 cells were seeded in 12-well plates. After 24 hours, the cells were treated with 0–50 μM of caffeate derivatives for 0–12 hours. The cells were then labeled with JC-1 according to the manufacturer's instructions. Thereafter, the cells were resuspended in adequate same solution and analyzed using a Flexstation 3 fluorescence plate reader (Molecular Devices) with an excitation wavelength of 560 nm and the emission wavelength of 595 nm for red fluorescence. Apoptotic cells will generate a lower reading of red fluorescence, and the changes in the mitochondria membrane potential ($\Delta\Psi m$) can be assessed by comparing the red fluorescence of untreated cells and cells treated with caffeate derivatives.

2.6. Western blot analysis

A549 cells (10^7 cells/10 cm dish) were incubated with 25 μM of caffeate derivatives for 0 hours, 1 hour, 3 hours, 6 hours, 9 hours, 12 hours, and 24 hours. Cells were collected and lysed in ice-cold lysis buffer to determine the protein concentration (Bio-Rad DC protein assay, Bio-Rad Laboratories, Hercules, CA, USA). The Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, -9, -3, PARP, and β-actin proteins were assessed in A549 cells. Total proteins (50–60 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 12% polyacrylamide gel. The proteins in the gel were transferred to a polyvinyldifluoride membrane and then blocked with 5% skim milk in PBST (0.05% v/v Tween-20 in phosphate buffered saline, pH 7.2) for 1 hour. Membranes were incubated with primary antibody (1:1000) at 4°C overnight and then with secondary antibody (1:2000) for 1 hour. Membranes were washed three times in PBST for 10 minutes between each step. The signal was detected using enhanced chemiluminescence (ECL; Perkin-Elmer Life Science, Boston, MA, USA).

2.7. Statistical analysis

Data are presented as the mean ± standard deviation for the indicated number of independently performed experiments. Significant differences ($p < 0.05$) between the groups were calculated using Student unpaired t test.

3. Results

3.1. Effects of caffeate derivatives on the viability of A549 cells

The chemical structures of octyl caffeate, phenylpropyl caffeate, and decyl caffeate are shown in Fig. 1. To assess whether these three caffeate derivatives (octyl caffeate, phenylpropyl caffeate, and decyl caffeate) could inhibit the viability of A549 human lung carcinoma, cells were treated with 0–100 μM caffeate derivatives for 0–48 hours, and cell viability was determined via the MTT assay. The effects of octyl caffeate, phenylpropyl caffeate, and decyl caffeate on cell viability in A549 cells are shown in Table 1. The results indicate that addition of octyl caffeate, phenylpropyl caffeate, and decyl caffeate to the growth medium decreased the viability of A549 cells ($p < 0.05$). The IC_{50} (a concentration at which cell viability is 50% of control) values of octyl caffeate, phenylpropyl caffeate, and decyl caffeate on A549 cells for 24 hours were 79.0 ± 1.0 μM, >100 μM, and 83.2 ± 1.5 μM, respectively. The IC_{50} values of octyl caffeate, phenylpropyl caffeate, and decyl caffeate on A549 cells for 48 hours were 54.2 ± 10.1 μM, 80.2 ± 1.3 μM, and 74.9 ± 2.1 μM, respectively. These results indicate that octyl caffeate and decyl caffeate have the greatest viability inhibition of A549 cells of all caffeate derivatives tested. Therefore, octyl caffeate and decyl caffeate were selected for all subsequent studies.

3.2. Effects of octyl caffeate and decyl caffeate on cell apoptosis in A549 cells

Programmed cell death is characterized by a specific sequence of morphology changes including cell shrinkage,

Table 1
Effects of octyl caffeate, phenylpropyl caffeate, and decyl caffeate on the cell viability of A549 human lung carcinoma cells.[a]

Cell viability (% of control)	Caffeate derivatives (μM)				
	0	10	25	50	100
24 h					
Octyl caffeate	100.0 ± 6.1	83.2 ± 1.5*	80.4 ± 3.7*	79.1 ± 1.5*	28.8 ± 2.3*
Phenylpropyl caffeate	100.0 ± 3.1	98.3 ± 6.9	105.1 ± 6.0	106.0 ± 6.5	50.9 ± 14.5*
Decyl caffeate	100.0 ± 7.3	86.5 ± 3.7*	88.1 ± 3.0*	78.0 ± 7.8*	36.0 ± 3.3*
48 h					
Octyl caffeate	100.0 ± 3.0	70.8 ± 0.8*	69.7 ± 4.1*	53.5 ± 6.4*	21.6 ± 0.5*
Phenylpropyl caffeate	100.0 ± 3.4	87.1 ± 1.0*	84.5 ± 4.5*	88.5 ± 3.4*	25.3 ± 1.2*
Decyl caffeate	100.0 ± 2.7	81.8 ± 1.8*	85.1 ± 1.2*	74.3 ± 4.2*	25.7 ± 0.3*

Data are presented as mean ± SD ($n = 3$).
*$p < 0.05$ indicates significant differences from the control group.
[a] Cells were treated with 0–100 μM caffeate derivatives for 24 hours and 48 hours.

membrane blebbing, chromatin condensation, and the formation of apoptotic bodies.[13] The effects of octyl caffeate and decyl caffeate on cell morphology in A549 cells are shown in Fig. 2. This cell morphology showed that cell shrinkage, membrane blebbing, and apoptotic body formation were observed when cells were treated with 50 μM of octyl caffeate and decyl caffeate (Fig. 2A). The nuclear morphology of untreated and treated cells is shown in Fig. 2B by PI staining. PI staining revealed that apoptotic bodies appeared when the cells were treated with 50 μM of octyl caffeate and decyl caffeate for 48 hours.

3.3. Effects of octyl caffeate and decyl caffeate on the mitochondria membrane potential ($\Delta\Psi m$) in A549 cells

Alterations in mitochondria function have been shown to play a crucial role in cell apoptosis or programmed cell death. As shown in Table 2, A549 cells showed significantly ($p < 0.05$) decreased mitochondria membrane potential when treated with 25 μM (6 hours and 12 hours) and 50 μM (12 hours) octyl caffeate. Treatment of A549 cells with 50 μM decyl caffeate for 6 hours and 12 hours caused the loss of mitochondria membrane potential ($p < 0.05$). Loss of mitochondria membrane potential is an early stage of apoptosis that may further activate the intrinsic pathways of apoptosis.

3.4. Octyl caffeate and decyl caffeate induce apoptosis in A549 cells

The effects of octyl caffeate and decyl caffeate on the expression of apoptotic proteins in A549 human lung carcinoma cells were measured by Western blot analysis. The effect of decyl caffeate on the levels of Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, -9, -3, and PARP in A549 cells for 0 hours, 1 hour, 3 hours, 6 hours, 9 hours, 12 hours, and 24 hours is shown in Fig. 3. The maximal levels of the Fas and FasL proteins occurred at 3 hours after being treated with octyl caffeate. However, octyl caffeate did not affect AIF expression when cells were treated with 25 μM for 0–24 hours. Octyl caffeate treatment (25 μM, 6–24 hours) resulted in a significant increase in the Apaf-1 level. The protein level of FLIP decreased after treatment of A549 cells with 25 μM of octyl caffeate for 6–24 hours. The effects of the compounds on the levels of pro- and anti-Bcl-2 family proteins were also examined. Treatment of A549 cells with octyl caffeate increased the protein levels of Bax and Bad and decreased that of Bcl-2. Furthermore, treatment of A549 cells with octyl caffeate caused the activation of caspase-12, -9, and -3, which are associated with the degradation of PARP. We also demonstrated that decyl caffeate stimulates an increase in the protein levels of Fas, FasL, and Apaf-1 and a decrease in FLIP (Fig. 4). Similar to octyl caffeate, treatment of A549 cells with decyl caffeate changed the levels of pro- and antiapoptotic Bcl-2 family members and induced the activation of caspase-12, -9, and -3, which was followed by the cleavage of PARP.

4. Discussion

Lung cancer is one of the most common types of cancer in the developed world and is the most lethal of all cancers worldwide. Caffeate derivatives such as CAPE have been reported to exhibit antioxidant, anti-inflammatory, and anticancer activities.[4] However, studies demonstrating the anticancer effects of caffeate derivatives, including octyl caffeate, phenylpropyl caffeate, and decyl caffeate, in human cancer cells remain inconclusive. Therefore, octyl caffeate, phenylpropyl caffeate, and decyl caffeate were selected for testing. The objective of this study was to investigate the effects of octyl, phenylpropyl, and decyl caffeates on induction of cell apoptosis and the apoptotic pathway in A549 human lung carcinoma cells. The induction of apoptosis can provide a therapeutic strategy for the treatment of cancer.[14] Apoptosis or programmed cell death is characterized by caspase-mediated specific morphological changes including cell shrinkage, chromatin condensation, nuclear DNA fragmentation, membrane blebbing, and the release of apoptotic bodies from apoptotic cells.[15] Many studies have indicated that caffeate derivatives induce apoptosis in human cancer cells, such as U937 human leukemia cells,[7] and SK-MEL-28 human melanoma cells.[10] However, it is still unclear whether caffeate derivatives induce apoptosis of human lung cancer cells.

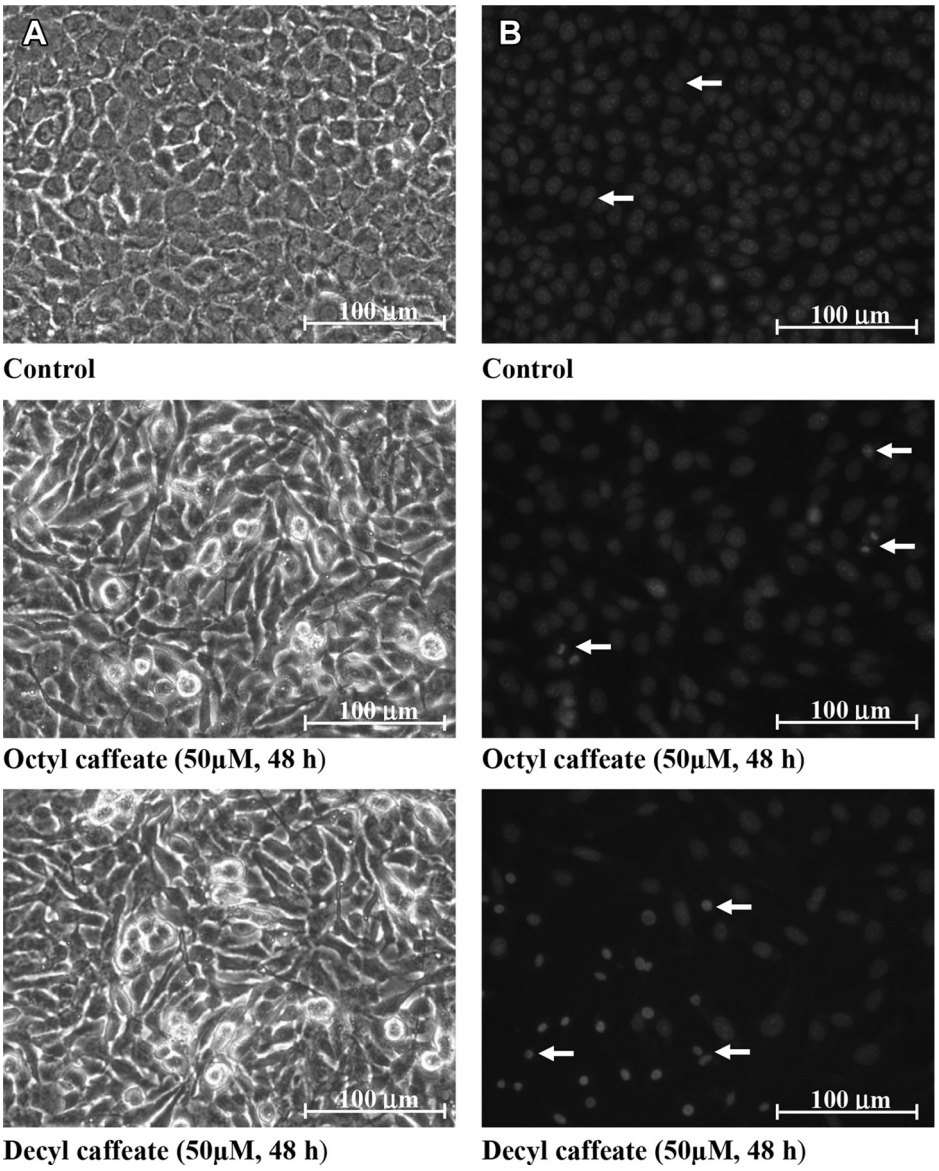


Fig. 2. Effects of octyl caffeate and decyl caffeate on cell morphology in A549 human lung carcinoma cells. (A) Unstained and (B) stained with propidium iodide. Cells were treated with 50 μM caffeate derivatives for 48 hours.

In this study, the results from an MTT assay demonstrated that octyl caffeate, phenylpropyl caffeate, and decyl caffeate significantly decreased the cell viability of A549 human lung carcinoma cells. The results suggest that these caffeate derivatives have cytocidal activity on A549 lung cancer cells. Octyl caffeate and decyl caffeate have higher cytocidal activity on A549 cells than phenylpropyl caffeate. Therefore, octyl caffeate and decyl caffeate were selected for PI nuclear staining, mitochondria membrane potential assay, and molecular mechanism studies. We further demonstrated that both octyl caffeate and decyl caffeate have the ability to induce apoptosis of A549 cells. PI staining showed that nuclear condensation and formation of apoptotic bodies occur when cells were treated with 50 μM octyl caffeate and decyl caffeate for 48 hours. Several indicators for apoptosis were also induced by octyl caffeate and decyl caffeate, e.g., loss of mitochondria membrane potential,

Table 2
Effects of octyl caffeate and decyl caffeate on mitochondrial membrane potential (ΔΨm) in A549 human lung carcinoma cells.[a]

ΔΨm (% of control)	Caffeate derivatives (μM)		
	0	25	50
6 h			
Octyl caffeate	100.0 ± 0.8	93.7 ± 1.0*	99.2 ± 0.8
Decyl caffeate	100.0 ± 0.9	103.9 ± 0.7	85.9 ± 0.4*
12 h			
Octyl caffeate	100.0 ± 0.9	93.8 ± 0.1*	90.5 ± 0.7*
Decyl caffeate	100.0 ± 0.9	101.1 ± 1.2	85.2 ± 0.8*

Data are presented as mean ± SD ($n = 3$).
*$p < 0.05$ indicates significant differences from the control group.
[a] Cells were treated with 0–50 μM caffeate derivatives for 6 hours and 12 hours.

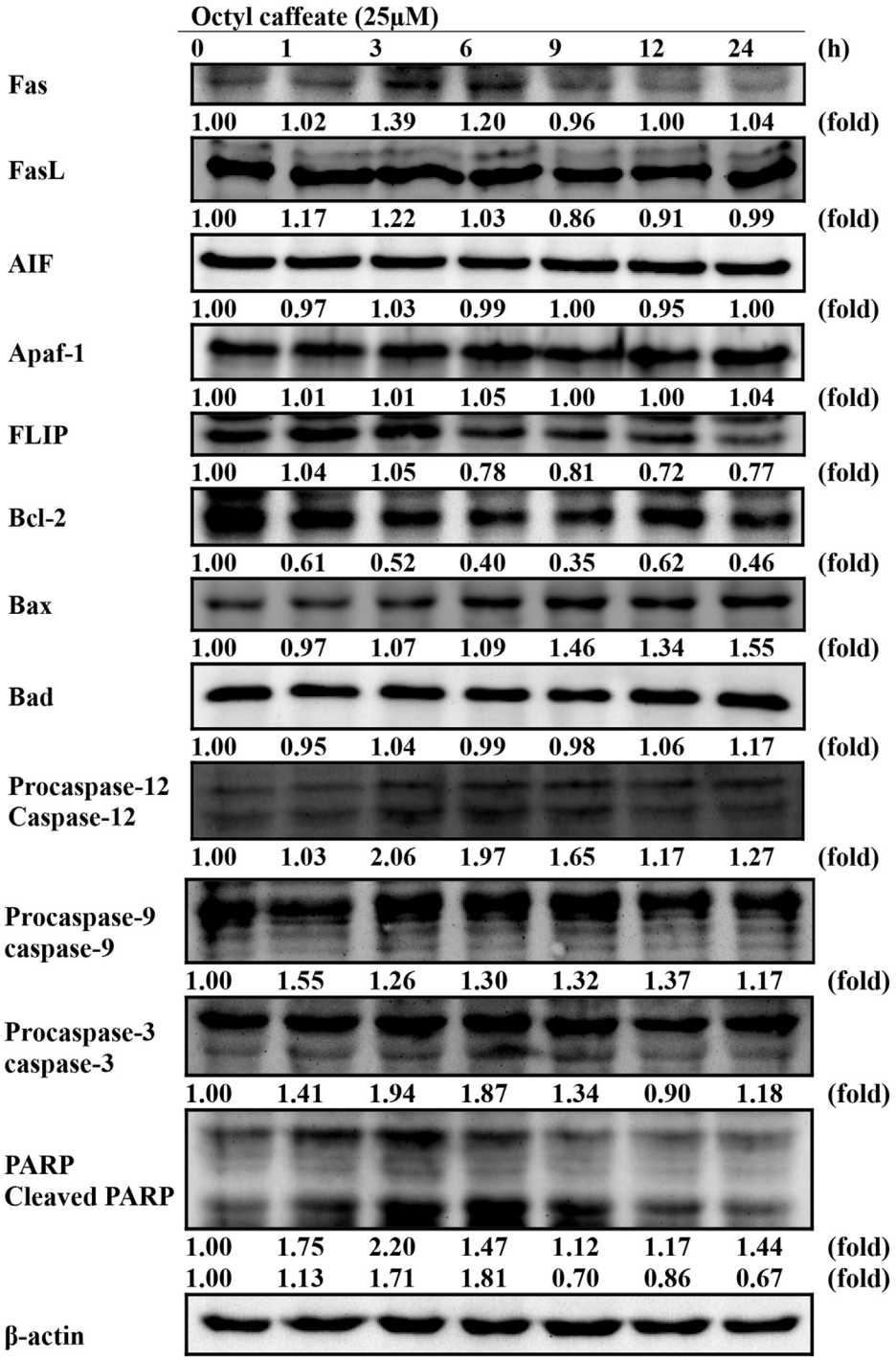


Fig. 3. Effect of octyl caffeate on protein levels of Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, -9, -3, and PARP in A549 human lung carcinoma cells. Cells were treated with 25 μM octyl caffeate for 0–24 hours. The relative level of expression of proteins was quantified densitometrically using Image Analysis Program LabWorks version 4.5 (UVP Imagining Company, Upland, CA, USA) and was calculated according to the β-actin reference bands. AIF = anti-apoptosis-inducing factor; Apaf-1 = anti-apoptotic protease activating factor-1; FLIP = Fas-associated death-domain-like IL-1β-converting enzyme-inhibitory protein; PARP = poly (ADP-ribose) polymerase.

activation of the caspases, and cleavage of PARP. All of these results suggest that these caffeate derivatives have therapeutic potential against lung cancer via induction of apoptosis, and octyl caffeate has more anticancer potential than decyl caffeate and phenylpropyl caffeate.

There are two major apoptotic pathways: the intrinsic (mitochondria-dependent) and the extrinsic (death receptor-dependent) signaling pathways.[16] The intrinsic pathway is triggered by non–receptor-mediated stimuli, which upregulate proapoptotic proteins (e.g., Bax and Bad) and downregulate antiapoptotic proteins (e.g., Bcl-2), and cause an increase in mitochondria permeability, a loss of mitochondria potential, and the release of cytochrome c. The released cytochrome c further binds to Apaf-1, ATP, and caspase-9 to

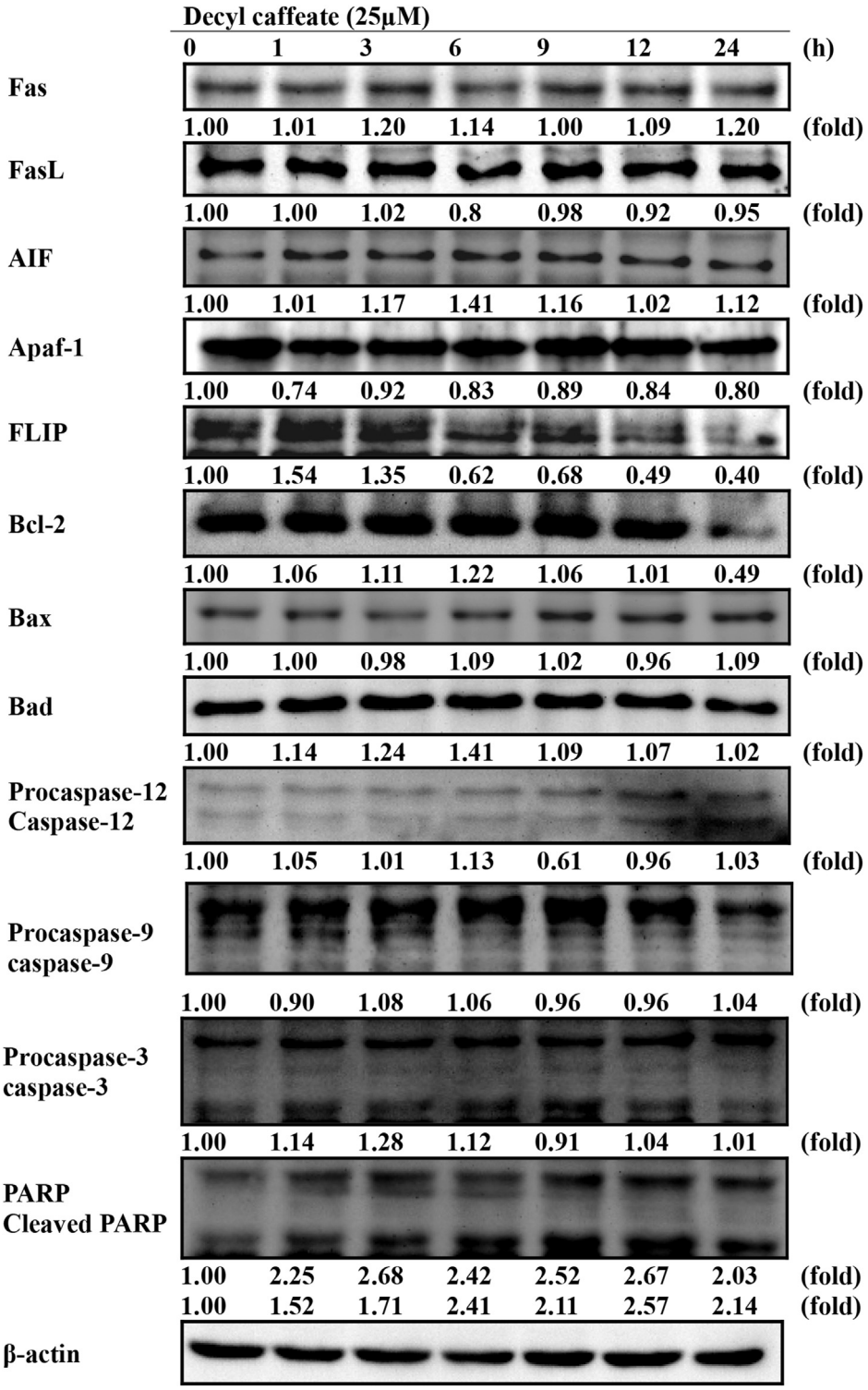


Fig. 4. Effect of decyl caffeate on protein levels of Fas, FasL, AIF, Apaf-1, FLIP, Bcl-2, Bax, Bad, caspase-12, -9, -3, and PARP in A549 human lung carcinoma cells. Cells were treated with 25 μM decyl caffeate for 0–24 hours. The relative level of expression of proteins was quantified densitometrically using LabWorks version 4.5 and was calculated according to the β-actin reference bands. AIF = anti-apoptosis-inducing factor; Apaf-1 = anti-apoptotic protease activating factor-1; FLIP = Fas-associated death-domain-like IL-1β-converting enzyme-inhibitory protein; PARP = poly (ADP-ribose) polymerase.

form apoptosomes, which in turn activates caspase-3 and initiates apoptotic degradation.[16–24] This study indicated that treatment of A549 cells with octyl caffeate and decyl caffeate decreased the mitochondria membrane potential, decreased the level of Bcl-2, and increased the levels of Bax, Bad, Apaf-1, caspase-3, and -9. Moreover, octyl caffeate and decyl caffeate also induced the cleavage of PARP. The results suggest that octyl caffeate and decyl caffeate induce

apoptosis through the mitochondria-dependent pathway in A549 cells.

By contrast, the extrinsic pathway involves the death-inducing signaling complex including Fas and its receptor Fas ligand (FasL), which serves an important role in regulating the induction of apoptosis in diverse cell types and tissues.[25] The binding of FasL to the Fas receptor activates caspase-8, which in turn activates caspase-3 to initiate apoptotic degradation.[16] In this study, the levels of Fas and FasL were increased by the octyl and decyl caffeates treatments, suggesting that the caffeate derivatives also induce apoptosis through the death receptor-dependent pathway in A549 cells.

In addition, FLIP, which structurally resembles caspase-8 except that it lacks proteolytic activity, can inhibit death receptor-mediated apoptosis.[26,27] We demonstrated that octyl caffeate and decyl caffeate stimulate a decrease in the protein level of FLIP, suggesting that these signal molecules may also be involved in the caffeate derivatives-mediated induction of apoptosis. Moreover, there also exists a mitochondria- and death receptor-independent apoptosis pathway, namely endoplasmic reticulum (ER)-induced apoptosis. The central player in the ER-induced apoptosis pathway is caspase-12 protein.[28] Activated caspase-12 can translate from the ER to the cytosol, where it directly cleaves procaspase-9 and, in turn, activates the effector caspase, i.e., caspase-3. In this study we found that the expression of caspase-12 was increased following exposure to caffeate derivatives, suggesting that the ER-induced apoptotic pathway is also involved in the induction of apoptosis by caffeate derivatives. Furthermore, it has been shown that apoptosis can also be induced through regulation of a caspase-independent mechanism, the AIF-mediated pathway.[29] In this study, the expression of AIF was not obviously increased following treatment with caffeate derivatives, suggesting that the AIF-mediated pathway is not involved in caffeate derivative-mediated induction of apoptosis in A549 cells. Zhou et al[30] indicated that TRIM29 and β-catenin may play an important role in the pathogenesis of lung cancer. However, further examination on the TRIM29 and β-catenin interactions is needed. Syntheses of octyl caffeate, phenylpropyl caffeate, and decyl caffeate were semi-derived from caffeic acid. Chemical properties of octyl caffeate, phenylpropyl caffeate, and decyl caffeate are as follows: (1) octyl caffeate: white solid, melting point 98–100°C, molecular weight 292.37, and calculated for $C_{17}H_{24}O_4$; (2) phenylpropyl caffeate: white solid, melting point 102–103°C, molecular weight 298.33, and calculated for $C_{18}H_{18}O_4$; (3) decyl caffeate: white solid, melting point 108–109°C, molecular weight 320.42, and calculated for $C_{19}H_{28}O_4$. Ujibe et al[7] found that octyl caffeate induced apoptosis in human leukemia U937 cells and is six-fold more potent than caffeic acid phenethyl ester (derived from caffeic acid). Other biological activities of derivatives of caffeic acid, Hsiao et al[31] indicated that octyl caffeate is a potent antioxidant in many caffeate derivatives. Chou et al[32] found that *N*-2-(4-bromophenyl) ethyl caffeamide inhibitory effect on MMP-9 expression via NF-κB in human monocytic cells. Weng et al[33] indicated that KS370G (caffeamide derivative) inhibited cardiac hypertrophy via modulations of p-ERK, p-AKT, and p-GSK3β in pressure-overload mice heart. Ho et al[34] found that caffeic acid phenethyl amide attenuates the glucose homeostasis in streptozotocin-induced type 1 diabetic rats.[3]

In conclusion, the present study showed that octyl caffeate and decyl caffeate induce apoptosis of A549 human lung carcinoma cells. Octyl caffeate and decyl caffeate also caused the loss of mitochondria membrane potential. Octyl caffeate and decyl caffeate induced apoptosis in A549 cells through many cellular effects: increased protein levels of Fas, FasL, and Apaf-1; decreased protein level of FLIP; altered levels of pro- and antiapoptotic Bcl-2 family members; activation of caspase-12, -9, and -3; and finally, cleavage of PARP. These results demonstrate that octyl caffeate and decyl caffeate induces cell apoptosis in A549 human lung carcinoma cells.

Acknowledgments

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