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ARTICLE

Effects of Polyphenolic Compounds on Tumor Necrosis Factor-α (TNF-α)-Induced Changes of Adipokines and Oxidative Stress in 3T3-L1 Adipocytes

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Over the last few decades, obesity has become a global epidemic in both developed and developing countries. Recent studies have indicated that obesity is closely associated with chronic inflammation characterized by abnormal levels of adipocytokines and inflammatory cytokines in adipocytes. The aim of this work was to study the effects of 21 polyphenolic compounds on tumor necrosis factor-α (TNF-α)-induced changes of adipokines and oxidative stress in 3T3-L1 adipocytes. The results showed that *p*-coumaric acid, quercetin, and resveratrol have greater inhibition ($p < 0.05$) of a TNF-α-induced increase in the production of interleukin-6 (IL-6) among 21 tested polyphenolic compounds. *p*-Coumaric acid, quercetin, and resveratrol demonstrated inhibitions of TNF-α-induced changes in levels of monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), and intracellular reactive oxygen species (ROS) in 3T3-L1 adipocytes. Furthermore, *p*-coumaric acid, quercetin, and resveratrol increased levels ($p < 0.05$) of secreted adiponectin, superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and glutathione *S*-transferase (GST) in TNF-α-treated 3T3-L1 adipocytes. These results indicate that the inhibition of TNF-α-induced changes of adipokines and oxidative stress by some polyphenolic compounds might have further implications in preventing obesity-related pathologies.

KEYWORDS: Polyphenolic compounds; adipocytes; adipokines; oxidative stress

INTRODUCTION

Obesity is associated with chronic inflammation and several obesity-related epidemic diseases, such as type-2 diabetes, cardiovascular diseases, insulin resistance, and certain cancers (*1*). The report indicated that increased pro-inflammatory factors, such as interleukin-6 (IL-6), resistin, and tumor necrosis factor-alpha (TNF-α), are observed in obesity-related diseases (*2*). IL-6 is expressed and secreted by macrophages and adipocytes and are reported to be linked to the development of obesity-related diseases, such as type-2 diabetes and insulin resistance (*3*). The role of monocyte chemoattractant protein-1 (MCP-1) in macrophages and adipocytes is important in regulating inflammation (*4*). Increased levels of plasminogen activator inhibitor-1 (PAI-1) are also observed in obese people and strongly correlated with obesity and insulin resistance (*5*). Previous studies have indicated that the levels of adiponectin are decreased in obese and insulin-resistant humans and mice (*6*, *7*). Obesity-related inflammatory responses are partially mediated by multiple cellular stresses, such as oxidative stress, endoplasmic reticulum (ER) stress, and hypoxia (*8*−*10*). These reports have shown that the intake of a high-fat diet enhanced oxidative stress and glutathione disulfide (GSSG) content; however, a high-fat diet reduced the level of glutathione (GSH) and activities of glutathione peroxidase (GPx), glutathione reductase (GRd), and glutathione *S*-transferase (GST) (*11*−*13*).

Flavonoids and phenolic acids are constituents of fruits, vegetables, and plant-derived foodstuffs, including tea, wine, and traditional Asian herbs. They are believed to have important antioxidant, anti-inflammatory, anti-cancer, anti-obesity, anti-HIV-1, and anti-thrombotic pharmacological properties (*14*). Numerous studies have demonstrated that phenolic substances, such as curcumin, naringenin chalcone, and resveratrol attenuate obesity-related inflammatory responses (*15*, *16*). Our previous study revealed that *o*-coumaric acid and rutin had the highest inhibition on levels of intracellular triglyceride and glycerol-3-phosphate dehydrogenase activity among 21 naturally occurring polyphenolic antioxidants in 3T3-L1 adipocytes (*17*). However, the effects of an *in vitro* screening model of naturally occurring polyphenolic antioxidants on TNF-α-induced changes of adipokines and oxidative stress in 3T3-L1 adipocyte still remain unclear.

The murine 3T3-L1 cell line has been widely used in adipose cell biology studies (*18*). Hence, we would like to investigate the

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effects of polyphenolic compounds on TNF-α-induced changes of adipokines and oxidative stress in 3T3-L1 adipocytes. Moreover, in the present study, changes in levels of IL-6, MCP-1, PAI-1, adiponectin, reactive oxygen species (ROS), superoxide dismutase (SOD), GSH, GPx, and GST as induced by TNF-α were measured in 3T3-L1 adipocytes, as well.

MATERIALS AND METHODS

Materials. Naringenin, rutin, hesperidin, resveratrol, naringin, quercetin, salicylic acid, *p*-hydroxybenzoic acid, protocatechuic acid, gentisic acid, gallic acid, vanillic acid, 3,4-dimethoxybenzoic acid, syringic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapinic acid, chlorogenic acid, TNF-α, dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), and insulin (INS) were obtained from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), bovine calf serum, and the antibiotic mixture (penicillin−streptomycin) were purchased from the Invitrogen Co. (Carlsbad, CA). All other chemicals are reagent-grade.

Cell Culture. 3T3-L1 pre-adipocytes (BCRC 60159) were purchased from the Bioresource Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsinchu, Taiwan, Republic of China). 3T3-L1 pre-adipocytes were planted in 6-well plates and maintained in DMEM supplemented with 10% bovine calf serum, 1.5 g/L sodium bicarbonate, and 100 units/mL penicillin−streptomycin at 37 °C in a humidified 5% CO_2 incubator. Adipocytic differentiation was induced by adipogenic agents (0.5 mM IBMX, 1 μM DEX, and 1 μM INS) that were added to the culture medium for 4 days. Afterward, the medium was placed in normal culture medium and was freshly replaced every 48 h. The cells were harvested 8 days from the initiation of the differentiation. 3T3-L1 adipocytes were pretreated with 0−100 μM polyphenolic compounds for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. The protein concentration of cell lysates was estimated with the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA), using bovine serum albumin as the standard. To determine the levels of intracellular ROS, 3T3-L1 adipocytes were treated with 50 μM *p*-coumaric acid, quercetin, and resveratrol in the presence or absence of TNF-α (5 ng/mL) for 0−6 h.

Measurement of Secreted IL-6. Secreted IL-6 levels were determined by a mouse IL-6 enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA) according to the procedure of the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm, and the concentration of IL-6 was calculated as picograms per milligram of protein.

Measurement of Secreted MCP-1. Secreted MCP-1 levels were determined by a mouse MCP-1 ELISA kit (RayBiotech, Norcross, GA) as specified by the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm, and the concentration of MCP-1 was calculated as picograms per milligram of protein.

Measurement of Secreted PAI-1. Secreted PAI-1 levels were determined by a PAI-1 ELISA kit (AssayPro, Winfield, MO) as specified by the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm, and the concentration of PAI-1 was calculated as nanograms per milligram of protein.

Measurement of Secreted Adiponectin. Secreted levels of adiponectin were determined by a mouse adiponectin/Acrp30 ELISA kit (R&D Systems, Minneapolis, MN) as specified by the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm, and the concentration of adiponectin was calculated as nanograms per milligram of protein.

Determination of Intracellular ROS Production in 3T3-L1 Adipocytes. Intracellular ROS production was measured using the oxidant-sensitive fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA). The cells were washed twice with phosphate-buffered saline (PBS) and stained with 20 μM DCFH-DA for 15 min at room temperature. The intracellular ROS production in 3T3-L1 adipocytes was determined using a FLUOstar galaxy fluorescence plate reader (BMG Lab Technologies, Ltd., Offenburg, Germany).

Determination of SOD Activity in 3T3-L1 Adipocytes. SOD activity was determined by a SOD assay kit (Cayman Chemical Company, Ann Arbor, MI) according to the procedure of the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm, and the SOD activity was calculated as milliunits per milligram of protein.

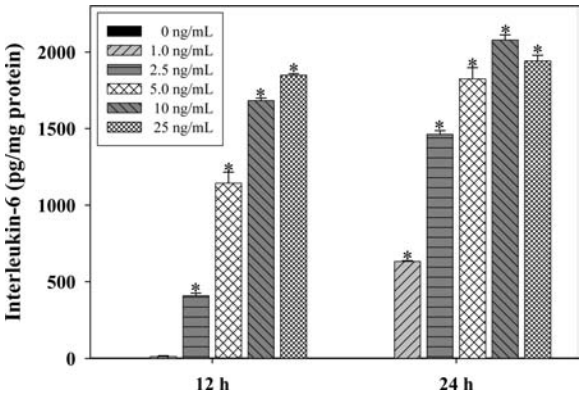

Figure 1. Effect of TNF-α on the secretion of IL-6 in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with 0−25 ng/mL TNF-α for 12 and 24 h, respectively, and then the level of IL-6 in the culture medium was assayed by ELISA. Reported values are the mean ± SD (n = 3). (*) $p < 0.05$ indicates significant differences from the control group.

Determination of GSH in 3T3-L1 Adipocytes. The GSH level was determined by a GSH assay kit (Cayman Chemical Company, Ann Arbor, MI) according to the procedure of the manufacturer. Absorbance was measured spectrophotometrically in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at 405 nm, and the concentration of GSH was calculated as nanomoles per milligram of protein.

Determinations of GPx and GST in 3T3-L1 Adipocytes. The GPx activity was determined according to the method by Lawrence and Burk (*19*). In total, a 100 μL cell lysate was mixed with 800 μL of a 100 mmol/L potassium phosphate buffer (pH 7.4) containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 1 mmol/L NaN_3, 0.2 mmol/L NADPH, 1 unit/mL GRd, and 1 mmol/L GSH. After 5 min, 2.5 mmol/L H_2O_2 (100 μL) was added to start the reaction. The absorbance change at 340 nm was recorded over the course of 3 min. The enzyme activity was calculated using an extinction coefficient of $E_{340} = 6220\ M^{-1}\ cm^{-1}$, and the result was expressed in units of nanomoles of NADPH per minute per milligram of protein.

The GST activity was determined according to the method by Habig et al. (*20*). Moreover, a 100 μL cell lysate was mixed well with 880 μL of a 100 mmol/L potassium phosphate buffer (pH 6.5) containing 100 mmol/L GSH and 50 mmol/L 1-chloro-2,4-dinitrobenzene (CDNB). The absorbance change at 340 nm was recorded over the course of 3 min. The enzyme activity was calculated using an extinction coefficient of $E_{340} = 9.6\ mM^{-1}\ cm^{-1}$, and the result was expressed in units of nanomoles of CDNB−GSH conjugate formed per minute per milligram of protein.

Statistical Analysis. Each experiment was performed in triplicate. The results were expressed as the mean ± standard deviation (SD). Statistical analyses were performed using SAS software. Analysis of variance was performed using analysis of variation (ANOVA) procedures. Significant differences ($p < 0.05$) between means were determined by Duncan's multiple range tests.

RESULTS

Effects of 21 Polyphenolic Compounds on the TNF-α-Induced Inflammatory Reaction in 3T3-L1 Adipocytes. The effect of TNF-α on the secretion of IL-6 in 3T3-L1 adipocytes is shown in **Figure 1**. Our results showed that, with TNF-α, 3T3-L1 adipocytes (0−25 ng/mL, 12−24 h) increased secretions of IL-6. 3T3-L1 adipocytes treated with the same level of TNF-α for 24 h expressed higher levels of IL-6 than those treated for 12 h. To further investigate whether TNF-α treatment affects inflammatory reaction, adipokines, and oxidative stress, 3T3-L1 adipocytes were pretreated with 0−100 μM polyphenolic compounds

Table 1. Effects of Polyphenolic Compounds on TNF-α-Induced IL-6 Secretion in 3T3-L1 Adipocytes

treatment[a]	IL-6 (pg/mg of protein)
control	0.3 ± 0.0
TNF-α	1078.6 ± 57.8[b]
hydroxybenzoic acids	
salicylic acid	561.1 ± 7.6[c]
p-hydroxybenzoic acid	568.6 ± 7.8[c]
protocatechuic acid	542.6 ± 14.9[c]
gentisic acid	567.4 ± 13.4[c]
gallic acid	631.8 ± 31.3[c]
vanillic acid	505.7 ± 24.9[c]
3,4-dimethoxybenzoic acid	579.8 ± 7.6[c]
syringic acid	525.0 ± 15.7[c]
hydroxycinnamic acids	
o-coumaric acid	610.6 ± 5.9[c]
m-coumaric acid	550.0 ± 21.0[c]
p-coumaric acid	458.0 ± 6.8[c]
caffeic acid	606.7 ± 15.9[c]
ferulic acid	575.5 ± 10.3[c]
sinapinic acid	651.9 ± 10.3[c]
flavonoids	
naringenin	787.4 ± 6.2[c]
rutin	609.0 ± 3.8[c]
hesperidin	727.3 ± 5.2[c]
quercetin	460.1 ± 28.5[c]
naringin	750.4 ± 11.7[c]
chlorogenic acid	508.2 ± 20.6[c]
resveratrol	150.5 ± 23.6[c]

[a] 3T3-L1 adipocytes were pretreated with 100 μM polyphenolic compounds for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. The level of IL-6 in the culture medium was assayed by ELISA. Data are expressed as the mean ± SD ($n = 3$). [b] $p < 0.05$ indicates significant differences from the control group. [c] $p < 0.05$ indicates significant differences from the TNF-α-treated group.

for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. As shown in **Table 1**, the IL-6 secretion was remarkably increased to 1078.6 ± 57.8 pg/mg of protein when the 3T3-L1 adipocytes were treated with TNF-α (5 ng/mL) for 12 h. These data also illustrated that *p*-coumaric acid, quercetin, and resveratrol cause greater inhibition on TNF-α-induced IL-6 secretions than other polyphenolic compounds. When the 3T3-L1 adipocytes were treated with 100 μM *p*-coumaric acid, quercetin, and resveratrol for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h, their IL-6 productions were 458.0 ± 6.8, 460.1 ± 28.5, and 150.5 ± 23.6 pg/mg of protein, respectively. Therefore, *p*-coumaric acid, quercetin, and resveratrol were selected for followup analyses. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced IL-6 production in 3T3-L1 adipocytes are shown in **Figure 2**. Our data indicated that *p*-coumaric acid, quercetin, and resveratrol (25−100 μM) significantly reduced TNF-α-induced IL-6 production in a dose-dependent manner.

Effects of *p*-Coumaric Acid, Quercetin, and Resveratrol on Levels of TNF-α-Induced Adipokines in 3T3-L1 Adipocytes. Figure 3A shows the effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced MCP-1 production in 3T3-L1 adipocytes treated with TNF-α. The MCP-1 secretion increased to 1346.4 ± 81.0 pg/mg of protein when 5 ng/mL TNF-α was added to 3T3-L1 adipocytes for 12 h. In untreated control cells, MCP-1 production was 815.3 ± 53.0 pg/mg of protein. On the basis of our data, *p*-coumaric acid, quercetin, and resveratrol also significantly reduced TNF-α-induced MCP-1 productions, except when 25 μM resveratrol was added to cell cultures. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced PAI-1 secretions in 3T3-L1 adipocytes are shown in **Figure 3B**. The PAI-1 secretion from 3T3-L1 adipocytes remarkably increased to 9.73 ± 0.57 ng/mg of protein when 5 ng/mL TNF-α was added to 3T3-L1 adipocytes for 12 h.

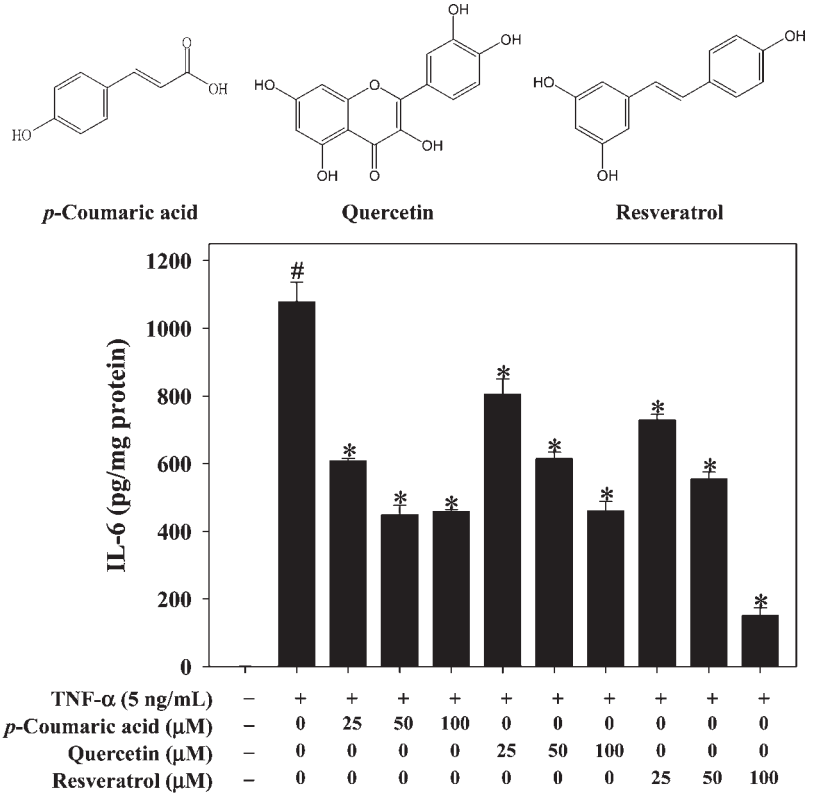

Figure 2. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced IL-6 production in 3T3-L1 adipocytes. 3T3-L1 adipocytes were pretreated with 0−100 μM polyphenolic compounds for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. The level of IL-6 in the culture medium was assayed by ELISA. Reported values are the mean ± SD ($n = 3$). (#) $p < 0.05$ indicates significant differences from the control group. (∗) $p < 0.05$ indicates significant differences from the TNF-α-treated group.

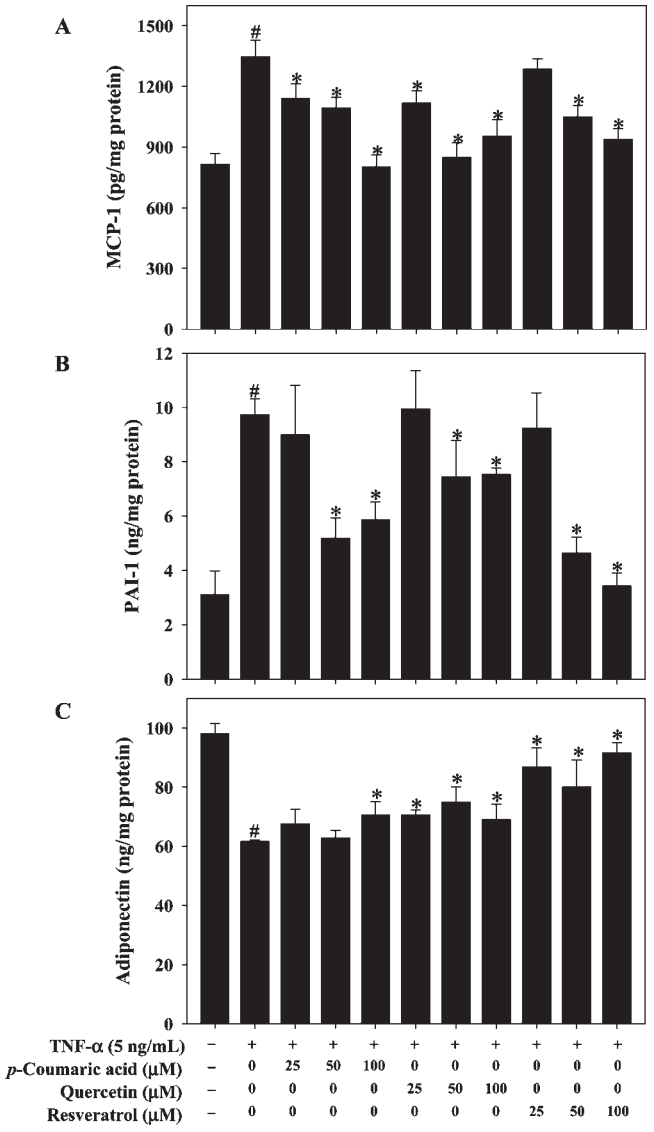

Figure 3. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced changes of (A) MCP-1, (B) PAI-1, and (C) adiponectin in 3T3-L1 adipocytes. 3T3-L1 adipocytes were pretreated with 0−100 μM polyphenolic compounds for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. The levels of MCP-1, PAI-1, and adiponectin in the culture medium were assayed by ELISA. Reported values are the mean ± SD (n = 3). (#) $p < 0.05$ indicates significant differences from the control group. (∗) $p < 0.05$ indicates significant differences from the TNF-α-treated group.

In untreated control cells, PAI-1 secretion was 3.12 ± 0.85 ng/mg of protein. Treatment of 3T3-L1 adipocytes with TNF-α caused lower PAI-1 productions when concentrations of *p*-coumaric acid, quercetin, or resveratrol were greater than 25 μM. Moreover, resveratrol significantly reduced TNF-α-induced PAI-1 production in a dose-dependent manner. Additionally, the adiponectin secretion was remarkably decreased to 61.6 ± 0.5 ng/mg of protein when TNF-α (5 ng/mL) was added to 3T3-L1 adipocytes for 12 h. In untreated control cells, adiponectin secretion was 98.0 ± 3.5 ng/mg of protein (**Figure 3C**). Quercetin and resveratrol (25−100 μM) significantly increased the TNF-α-induced change of adiponectin secretions. However, the increased adiponectin secretion in 3T3-L1 adipocytes was only obtained when 3T3-L1 adipocytes were pretreated with 100 μM *p*-coumaric acid.

Effects of *p*-Coumaric Acid, Quercetin, and Resveratrol on TNF-α-Induced Oxidative Stress in 3T3-L1 Adipocytes. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced

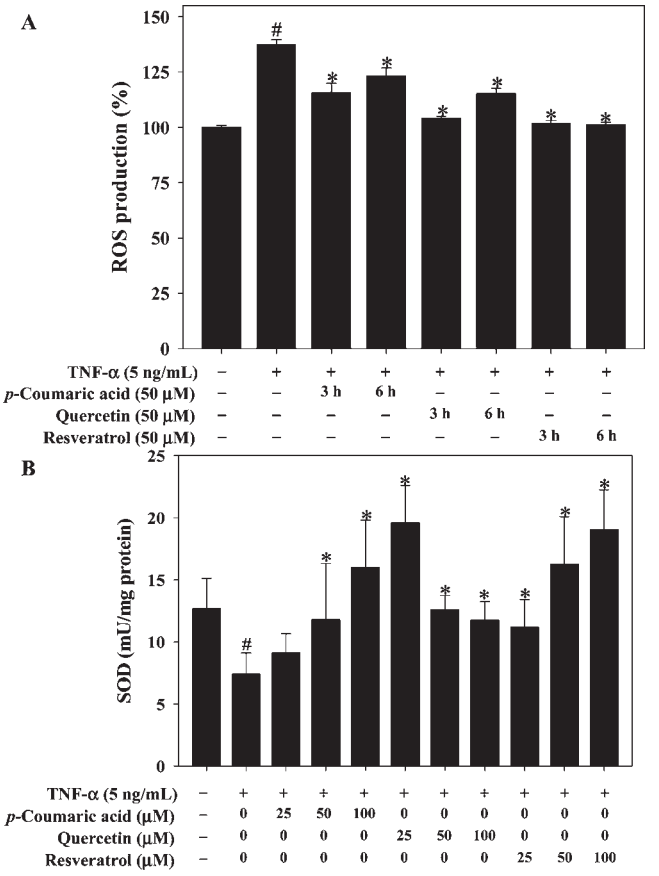

Figure 4. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced changes of (A) ROS and (B) SOD activity in 3T3-L1 adipocytes. 3T3-L1 adipocytes were pretreated with 0−100 μM polyphenolic compounds for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. Reported values are the mean ± SD (n = 3). (#) $p < 0.05$ indicates significant differences from the control group. (∗) $p < 0.05$ indicates significant differences from the TNF-α-treated group.

ROS production in 3T3-L1 adipocytes are shown in **Figure 4A**. *p*-Coumaric acid, quercetin, and resveratrol significantly suppressed the TNF-α-induced ROS production. As shown in **Figure 4B**, the SOD activity was remarkably decreased to 7.4 ± 1.7 mU/mg of protein when TNF-α (5 ng/mL) was added to 3T3-L1 adipocytes for 12 h. In untreated control cells, SOD activity was 12.7 ± 2.5 mU/mg of protein. Quercetin and resveratrol significantly increased SOD activity in TNF-α-treated 3T3-L1 adipocytes; however, an increased SOD activity was not observed until it was pretreated with 25 μM *p*-coumaric acid. In addition, effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced changes of GSH, GPx, and GST in 3T3-L1 adipocytes are shown in **Figure 5**. The GSH content was remarkably decreased to 4.11 ± 0.02 nmol/mg of protein when TNF-α (5 ng/mL) was added to 3T3-L1 adipocytes for 12 h. In untreated control cells, the GSH content was 6.92 ± 0.25 nmol/mg of protein (**Figure 5A**). Treatment of 3T3-L1 adipocytes with 25−100 μM *p*-coumaric acid, quercetin, or resveratrol significantly decreased the GSH levels depleted by TNF-α. Quercetin significantly increased GPx and GST activities in TNF-α-treated 3T3-L1 adipocytes; however, increased GPx and GST activities were not observed until they were pretreated with 25 μM *p*-coumaric acid and resveratrol (panels **B** and **C** of **Figure 5**).

DISCUSSION

TNF-α acts as a potent inducer for cell proliferation, apoptosis, inflammatory reaction, and lipid metabolism (*21*). Similarly,

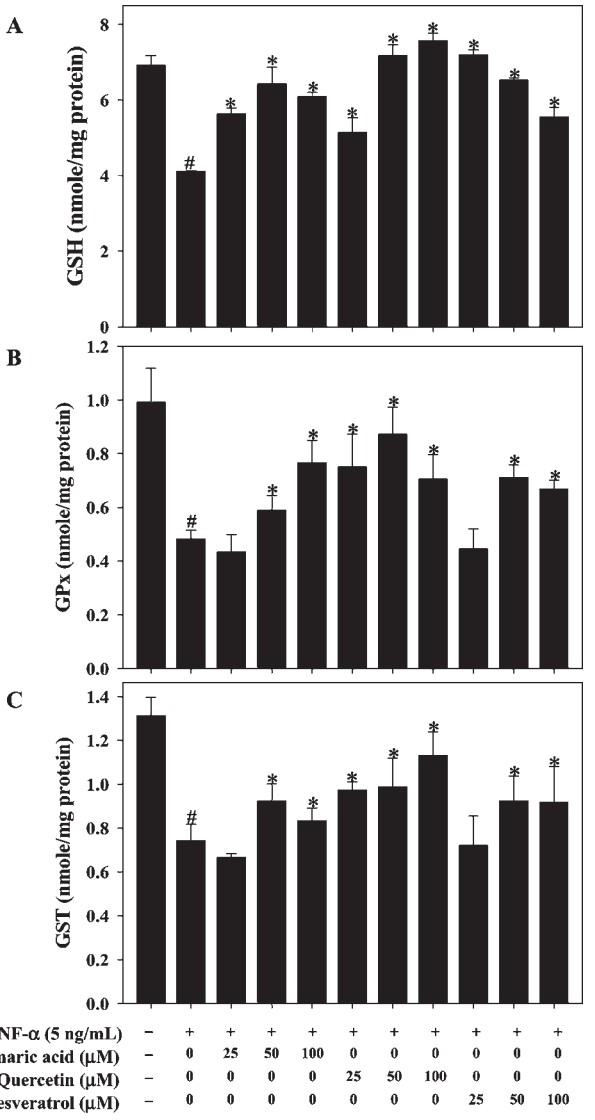

Figure 5. Effects of *p*-coumaric acid, quercetin, and resveratrol on TNF-α-induced changes of (A) GSH, (B) GPx, and (C) GST in 3T3-L1 adipocytes. 3T3-L1 adipocytes were pretreated with 0−100 μM polyphenolic compounds for 24 h and then exposed to TNF-α (5 ng/mL) for 12 h. Reported values are the mean ± SD (n = 3). (#) $p < 0.05$ indicates significant differences from the control group. (∗) $p < 0.05$ indicates significant differences from the TNF-α-treated group.

our data demonstrated that TNF-α-treated 3T3-L1 adipocytes (0−25 ng/mL, 12−24 h) significantly increased the secretion of IL-6 (**Figure 1**). Our data indicated that the secretion of IL-6 from 3T3-L1 adipocytes treated with TNF-α (5 ng/mL) for 12 h was 1143 pg/mg of protein, which was similar to a previous study by Araki et al. (*22*). Therefore, 5 ng/mL TNF-α for 12 h was selected for followup analyses. Samad et al. (*23*) indicated that the level of TNF-α was increased in obesity and was strongly correlated with high levels of adipokines in plasma and adipose tissue. On the basis of the present study, some potential polyphenolic compounds indeed improved the TNF-α-induced changes of inflammatory reaction, adipokines, and oxidative stress. *p*-Coumaric acid, quercetin, and resveratrol (100 μM) have the highest inhibitions of IL-6 secretion from TNF-α-stimulated 3T3-L1 adipocytes among 21 polyphenolic compounds (**Table 1** and **Figure 2**). Our previous study also illustrated that the cell numbers of 3T3-L1 adipocytes were not affected even with treatement consisting of 250 μM polyphenolic compounds (*17*). Thus, the inhibitory effects of 21 polyphenolic compounds in TNF-α-induced 3T3-L1 adipocytes are not attributed to cytotoxic effects. Ahn et al. (*24*) reported that resveratrol can attenuate IL-6, PAI-1, and adiponectin levels in 3T3-L1 adipocytes when added with TNF-α. Zhu et al. (*25*) indicated that resveratrol inhibits MCP-1 production and gene expression in 3T3-L1 adipocytes treated with TNF-α. Interestingly, our data are also in accordance with their data and show that not only resveratrol but also *p*-coumaric acid and quercetin have inhibitory effects on MCP-1 production in TNF-α-stimulated 3T3-L1 adipocytes (**Figure 3A**). MCP-1 is mainly expressed from endothelial cells, macrophages, and adipose tissues. Adipocyte-derived MCP-1 caused macrophage infiltration in adipose tissue and increased the production of pro-inflammatory cytokines (such as TNF-α), followed by the dysfunction of adipocytes (*26*). The present study also indicated that *p*-coumaric acid, quercetin, and resveratrol significantly inhibited TNF-α-induced PAI-1 production in 3T3-L1 adipocytes (**Figure 3B**). Adiponectin is produced and secreted exclusively from adipocytes and plays important roles in anti-atherogenesis, anti-inflammation, and anti-diabetes (*27*). Furthermore, *p*-coumaric acid, quercetin, and resveratrol significantly inhibited TNF-α-induced suppression of the adiponectin production from 3T3-L1 adipocytes (**Figure 3C**).

Obesity has been confirmed to be one of the conditions that decrease antioxidant defenses (*12*, *28*). The roles of antioxidants in enzymatic and non-enzymatic protections against oxidative-stress-induced toxicity have been discussed (*29*). Our results also showed that *p*-coumaric acid, quercetin, and resveratrol retard TNF-α-induced increased production of intracellular ROS in 3T3-L1 adipocytes (**Figure 4A**). Araki et al. (*22*) indicated that 3T3-L1 adipocytes pretreated with 10 ng/mL TNF-α had lower antioxidant enzyme activities (SOD, MnSOD, and catalase) but 20 mM *N*-acetylcysteine (NAC) significantly restored the decreased GSH concentration in 3T3-L1 adipocytes and expressed similar levels of intracellular ROS as adipocytes treated with TNF-α. Hence, it is speculated that *p*-coumaric acid, quercetin, and resveratrol significantly increased the SOD activity (**Figure 4B**), which may decrease the depletion of GSH content and the activities of GPx and GST in 3T3-L1 adipocytes treated with TNF-α (**Figure 5**). NAC, a well-known antioxidant, can normalize TNF-α-induced changes of IL-6, PAI-1, and adiponectin in 3T3-L1 adipocytes. Thus, NAC may be able to improve the obesity-related abnormal adipocytokine metabolism (*22*). NAC also ameliorated TNF-α-induced oxidant−antioxidant imbalance in adipocytes. Hence, naturally occurring polyphenolic antioxidants (*p*-coumaric acid, quercetin, and resveratrol) can also be characterized to have similar functionalities as NAC. These polyphenolic compounds significantly inhibited TNF-α-induced oxidative stress in 3T3-L1 adipocytes.

In conclusion, *p*-coumaric acid, quercetin, and resveratrol (50 μM) are able to inhibit productions of IL-6, MCP-1, PAI-1, and ROS in TNF-α-treated 3T3-L1 adipocytes. They also significantly alleviate the decreases of adiponectin, GSH, GPx, and GST, as well as SOD activity in TNF-α-treated 3T3-L1 adipocytes. This study might be the first investigation to demonstrate that *p*-coumaric acid, quercetin, and resveratrol can decrease TNF-α-induced oxidative stress in 3T3-L1 adipocytes. We would further investigate the *in vivo* inhibitory effects of these compounds on inflammatory reactions, adipokines, and oxidative stress, as well as their molecular mechanisms.

ABBREVIATIONS USED

DEX, dexamethasone; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; GPx, glutathione peroxidase; GRd, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; GST,

glutathione *S*-transferase; IBMX, 3-isobutyl-1-methylxanthine; IL-6, interleukin-6; INS, insulin; MCP-1, monocyte chemoattractant protein-1; NAC, *N*-acetylcysteine; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

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