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ORIGINAL CONTRIBUTION

Grape seed extract ameliorates tumor necrosis factor-α-induced inflammatory status of human umbilical vein endothelial cells

Chia-Lun Chao · Nen-Chung Chang · Ching-Sung Weng · Kueir-Rarn Lee · Shung-Te Kao · Jiin-Chyr Hsu · Feng-Ming Ho

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Abstract
Background Inflammation has played a key role in the causation of atherosclerosis. However, the effects of grape seed extract (GSE) on the pro-inflammatory intracellular signaling, enzyme activity, and inflammatory mediators of endothelial cells have not been sufficiently studied, and less information exists on the comparison between GSE and vitamin C, a well-known antioxidant compound, on their anti-inflammatory properties.
Purpose We investigated the effects of GSE and vitamin C on the cell viability, oxidative stress, monocyte adhesion, the expression of nuclear factor-κB inhibitor (IκB), intercellular adhesion molecule-1 (ICAM-1) and cyclooxygenase-2 (COX-2), and the production of prostaglandin E_2 (PG E_2) in TNF-α-treated human umbilical vein endothelial cells (HUVECs).
Methods Cell viability was measured by MTT assay. The adhesion of THP-1 to HUVECs was evaluated by cell adhesion assay. The oxidized nucleoside 8-hydroxydeoxyguanosine (8-OHdG) (an indicator of oxidative damage to DNA), ICAM-1, and PG E_2 were measured by ELISA. IκB and COX-2 expression were evaluated by western blot analysis.
Results TNF-α (10, 20, and 50 ng/mL), GSE (50 and 200 μg/mL), or vitamin C (100 μM) did not affect cell viability. GSE (50–100 μg/mL) attenuated TNF-α (20 ng/mL)-induced 8-OHdG production, THP-1 adhesion, the expression of IκB degradation, ICAM-1 and COX-2, and the production of PGE_2 in a dose-dependent manner. Vitamin C (100 μM) also showed significant antioxidative and anti-inflammatory effects.
Conclusions GSE effectively ameliorates TNF-α-induced inflammatory status of HUVECs. The findings of the present study suggest that consumption of GSE may be beneficial to inflammatory atherosclerosis.

Keywords Inflammation · Grape seed extract · Adhesion · Cyclooxygenase-2 · Prostaglandin E_2 · Endothelial cell

C.-L. Chao · J.-C. Hsu · F.-M. Ho (✉)
Division of Cardiology, Department of Internal Medicine, Taoyuan General Hospital, Department of Health, Executive Yuan, 1492 Chung-Shan Road, Taoyuan, Taiwan
e-mail: heart@mail.tygh.gov.tw; HoFM888@yahoo.com.tw

C.-L. Chao
Division of Cardiology, Department of Internal Medicine, National Taiwan University College of Medicine and National Taiwan University Hospital, Taipei, Taiwan

N.-C. Chang
Department of Internal Medicine, School of Medicine, Taipei Medical University, Taipei, Taiwan

C.-S. Weng
Department of Biomedical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan

K.-R. Lee
R&D Center for Membrane Technology and Department of Chemical Engineering, Chung Yuan Christian University, Taoyuan, Taiwan

S.-T. Kao · F.-M. Ho
Graduate Institute of Chinese Medical Science, College of Chinese Medicine, China Medical University, Taichung, Taiwan

Introduction

Inflammatory process has been found to play a key role in the causation of atherosclerosis, including initiation, progression (atheroma formation), and plaque rupture [1, 2]. The formation of atherosclerosis starts with the adherence of leukocytes to the vascular endothelial cells mediated by

adhesion molecules, such as P-selectin, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1). These molecules can be expressed by endothelial cells upon proatherogenic stimuli, including oxidized low-density lipoprotein, oxygen free radicals, as well as pro-inflammatory cytokines secreted by macrophages and T cells, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) [3, 4]. Moreover, cytokines can activate pro-inflammatory intracellular signaling, such as nuclear factor-kappa B (NF-κB) [5, 6], and stimulate the expression of cyclooxygenase-2 (COX-2) and production of prostaglandin E_2 (PG E_2), which leads to further cell adhesion [7], increase in endothelial permeability [8, 9], and instability of plaque [10].

Recently, clinical studies have demonstrated that systemic markers of inflammation are strong predictors of cardiovascular disease [2, 11, 12]. Vitamin C, which is a well-known strong antioxidant and also has anti-inflammatory properties, has been widely used to improve atherosclerosis [13, 14]. However, the results of the clinical studies were disappointing [15]. In contrast, several epidemiological studies have shown an inverse correlation between the dietary consumption of flavonoids and mortality from cardiovascular disease [16, 17]. Like vitamin C, flavonoids play an important role in the scavenging of oxygen free radicals and inhibition of inflammation [14]. Flavonoids, accounting for a major portion of polyphenols, are present abundantly in fruits, vegetables, nuts, and seeds and divided into six major subgroups: chalcones, flavonols, flavanone, flavones, anthocyanidins, and isoflavonoids [18]. After emergence of French paradox [19], grape seed extract (GSE), a popular commercial product that contains high quantity of flavonoids, especially anthocyanidins, has prompted the research interest in its effects on vascular protection [14, 20]. However, the effects of GSE on the pro-inflammatory intracellular signaling, enzyme activity, and inflammatory mediators of human umbilical vein endothelial cells (HUVECs) have not been sufficiently studied, and less information exists on the comparison between GSE and vitamin C on their anti-inflammatory properties.

In the present study, we investigated the effects of GSE and vitamin C on the cell viability, oxidative stress, monocyte adhesion, the expression of nuclear factor-κB inhibitor (IκB), ICAM-1 and COX-2, and the production of PGE_2 in TNF-α-treated HUVECs.

Methods

Cell culture of HUVECs

Human umbilical vein endothelial cells (HUVECs) were cultured as previously described [21]. Cells were seeded at a density of 1×10^5 per 75-cm^2 flask in medium 199 (Gibco, Grand Island, New York, USA), supplemented with 20 mM HEPES, 100 μg/mL endothelial cell growth substance (Collaborative Research Inc, Waltham, MA), and 20% fetal calf serum (Gibco, Grand Island, New York, USA). The cultures were maintained at 37 °C with a gas mixture of 5% CO_2 and 95% air. Subcultures were performed with trypsin–EDTA. All media were supplemented with 5 U/mL heparin, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin. Medium was refreshed every third day. The endothelial cells were identified by the presence of factor VIII-related antigen (Histoset Kit, Immunolok, Carpinteria, CA, USA) and a typical "cobblestone" appearance. Endothelial cells of the third to fifth passages in the actively growing condition were used for experiments.

Cell culture of THP-1 cells

THP-1 monocytes (ATCC, Manassas, VA, USA) were cultured in RPMI 1640 (Gibco, Grand Island, New York, USA) containing 10% fetal bovine serum, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, and 2 mM L-glutamine, pH 7.2. The cultures were maintained at 37 °C with a gas mixture of 5% CO_2 and 95% air.

MTT assay for cell viability

Cell viability was measured by a quantitative colorimetric assay using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), which shows mitochondrial activity of living cells. Cells in a 24-well plate were treated with TNF-α (Sigma Chemical Co., St. Louis, MO, USA) (10–20–50 ng/mL), GSE (Puritan's Pride, Oakdale, NY, USA) (50–200–400 μg/mL), or vitamin C (Sigma–Aldrich, St. Louis, MO, USA) (100 μM) for 24 h and then incubated with MTT for 4 h. The amount of MTT product was then determined by measuring the absorbance at 590 nm using an ELISA reader.

Cell adhesion assay

HUVECs were cultured in a 48-well plate containing M199 with 10% fetal bovine serum and treated with TNF-α (20 ng/mL) alone or in the presence of GSE (50–100 μg/mL) or vitamin C (100 μM) for 6 h. THP-1 cells were labeled with 2′,7′-bis-(carboxyethyl)-5(6′)-carboxyfluorescein acetoxymethyl ester (BCECF/AM) (Sigma–Aldrich, St. Louis, MO, USA) for 1 h and then seeded onto confluent HUVECs. The number of adherent THP-1 cells was measured by an ELISA reader with 485 nm excitation and 535 nm emission.

ELISA for 8-OHdG, ICAM-1, and PGE_2

In experiments, HUVECs were treated with TNF-α (20 ng/mL) alone or in the presence of GSE (50–100 μg/mL) or vitamin C (100 μM) for 6 h. Then supernatants were collected for the detection of 8-OHdG (Cosmo Bio Company LTD., Tokyo, Japan), ICAM-1 (Bender MedSystems Inc, Burlingame, CA, USA), and PGE_2 (NEOGEN Corporation, Lexington, KY, USA) using ELISA according to the manufacturers' instructions.

Western blot analysis for IκB-α and COX-2

The IκB-α and COX-2 antibodies (Upstate Biotechnology, Lake City, NY, USA) were used for analyses. HUVECs were washed twice with ice-cold phosphate-buffered saline and lysed in 200 μL of homogenization buffer (10 mM Tris–HCl at pH 7.4, 2 mM EDTA, 1 mM EGTA, 50 mM NaCl, 1% TritonR X-100, 50 mM NaF, 20 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 1:100 v/v proteinase inhibitor cocktail) at 4 °C for 30 min. Cell lysates were then ultracentrifuged at 12,000 g for 30 min at 4 °C, and the supernatants were used as the cell extracts. The cell extracts were subjected to 12% SDS–polyacrylamide gel and then transferred to the polyvinylidene fluoride (PVDF) membrane with a semi-dry blot apparatus (Bio-Rad) at 3 mA/cm^2 of the gel in transfer buffer (25 mM Tris at pH 8.3, 192 mM glycine, and 20% methanol) at room temperature for 30 min. The free protein-binding sites on the PVDF membrane were blocked by incubation with 5% nonfat milk in Tris/Tween-buffered saline (TTBS) (20 mM Tris at pH 7.4, 0.15 M NaCl, and 0.2% Tween-20) at 25 °C for 2 h. The membrane was immunoblotted with 0.1 μg/mL primary antibody in TTBS buffer containing 3% nonfat milk at 4 °C overnight and then with secondary antibody conjugated with peroxidase (1:1,000) at 25 °C for 1 h. Immunoblots were developed using an enhanced chemiluminescence system. The luminescence was visualized on X-ray film.

Data analysis

Data were obtained from at least three separate experiments and presented as mean ± SEM. All statistical data were obtained by analysis of variance followed by Student's *t* test. A *P* value <0.05 was considered statistically significant.

Results

Effect of TNF-α, GSE, and vitamin C on cell viability

TNF-α (10, 20, and 50 ng/mL), GSE (50 and 200 μg/mL), or vitamin C (100 μM) alone did not affect the cell viability of HUVECs. GSE (400 μg/mL) alone reduced the cell viability (Fig. 1).

Effect of GSE and vitamin C on 8-OHdG production

8-OHdG, a good indicator of ROS-induced DNA damage, was employed to detect oxidative stress. The 8-OHdG production in HUVECs was significantly increased by TNF-α (20 ng/mL). The TNF-α-induced 8-OHdG production was significantly reduced by GSE (50 μg/mL) and vitamin C (100 μM) (Fig. 2).

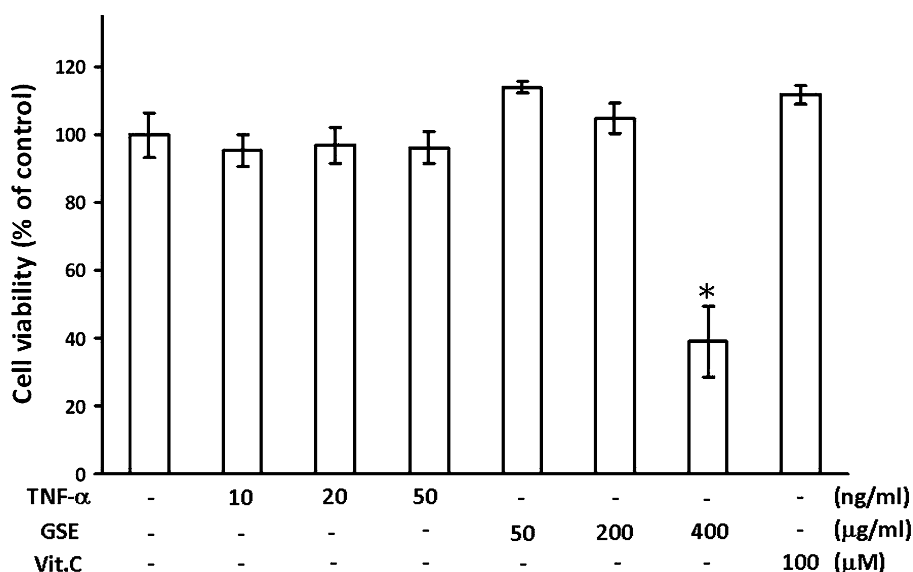

Fig. 1 Effect of TNF-α, GSE, and vitamin C on cell viability in HUVECs. TNF-α (10, 20, and 50 ng/mL), GSE (50 and 200 μg/mL), and vitamin C (100 μM) did not affect the cell viability. GSE (400 μg/mL) alone reduced the cell viability. Cells were treated with TNF-α, GSE, or vitamin C for 24 h. The cell viability was determined by MTT assay as described in "Methods". Data are mean ± SEM ($n = 4$). *$P < 0.05$ vs. control

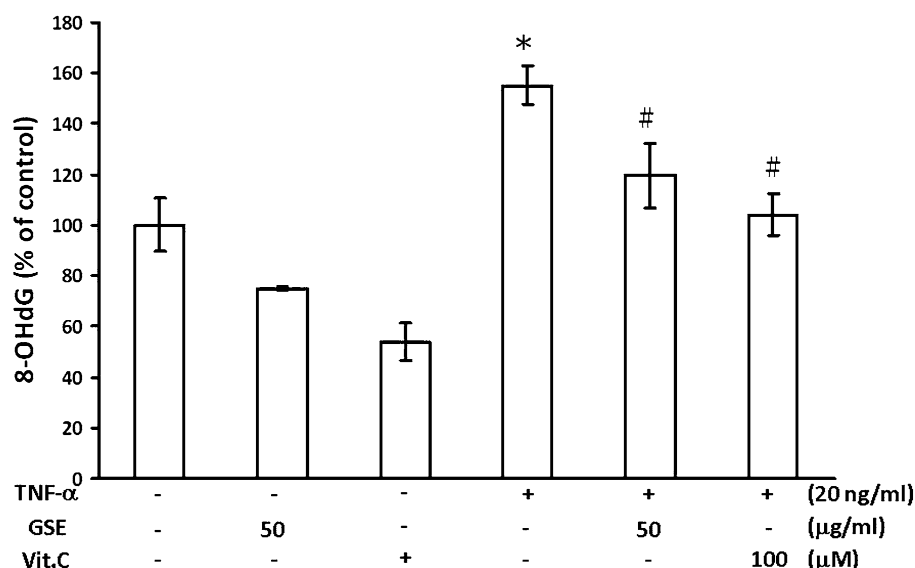

Fig. 2 Effect of GSE and vitamin C on 8-OHdG production in HUVECs. The 8-OHdG production in HUVECs was significantly increased by TNF-α (20 ng/mL). The TNF-α-induced 8-OHdG production was reduced by GSE (50 µg/mL) and vitamin C (100 µM). Cells were treated with TNF-α alone or in the presence of GSE or vitamin C for 6 h. The measurement of 8-OHdG was performed by ELISA as described in "Methods". Data are mean ± SEM ($n = 4$). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. TNF-α (20 ng/mL)

Effect of GSE and vitamin C on THP-1 adhesion

TNF-α (20 ng/mL) significantly increased the adhesion of THP-1 to HUVECs. The TNF-α-induced adhesion of THP-1 to HUVECs was decreased by GSE (50 and 100 µg/mL) in a dose-dependent manner. The inhibitory effect of vitamin C (100 µM) on TNF-α-induced THP-1 adhesion to HUVECs was also prominent (Fig. 3).

Effect of GSE and vitamin C on IκB expression

TNF-α (20 ng/mL) caused a significant decrease in IκB expression in HUVECs. GSE (50 and 100 µg/mL) showed a dose-dependent manner in attenuating the degradation of IκB-α. The attenuating effect of vitamin C (100 µM) on TNF-α-induced IκB degradation was also prominent (Fig. 4).

Effect of GSE and vitamin C on ICAM-1 expression

TNF-α (20 ng/mL) significantly increased ICAM-1 expression in HUVECs. The TNF-α-induced ICAM-1 expression was significantly reduced by GSE (50 µg/mL) and vitamin C (100 µM) (Fig. 5).

Effect of GSE and vitamin C on COX-2 expression

TNF-α (20 ng/mL) caused a significant increase in COX-2 expression in HUVECs. GSE (50 and 100 µg/mL) showed a dose-dependent manner in inhibiting COX-2

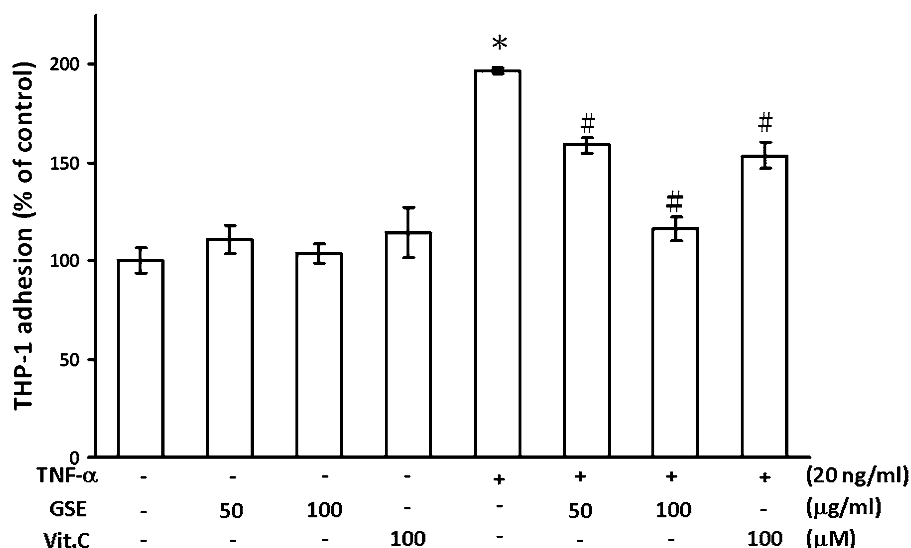

Fig. 3 Effect of GSE and vitamin C on THP-1 adhesion to HUVECs. TNF-α (20 ng/mL) increased the adhesion of THP-1 to HUVECs. The TNF-α-induced THP-1 adhesion was decreased by GSE in a dose-dependent manner. Cells were treated with TNF-α alone or in the presence of GSE or vitamin C for 6 h. The THP-1 adhesion was determined by cell adhesion assay as described in "Methods". Data are mean ± SEM ($n = 4$). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs TNF-α (20 ng/mL)

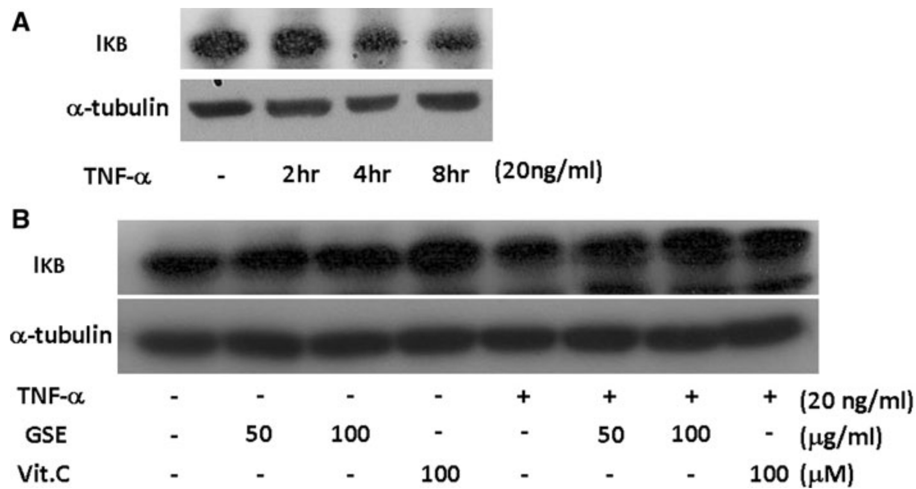

Fig. 4 Effect of GSE and vitamin C on IκB expression in HUVECs. **a** After treatment of TNF-α (20 ng/mL), the protein level of IκB rapidly decreased. **b** The expression of IκB was decreased by TNF-α (20 ng/mL) and increased by GSE (50 and 100 μg/mL) and vitamin C (100 μM). GSE showed a dose-dependent manner in attenuating the TNF-α-induced degradation of IκB. Cells were treated with TNF-α alone or in the presence of GSE or vitamin C for 6 h. Western blotting to identify IκB was performed as described in "Methods". The data are from a representative experiment of three experiments with similar results

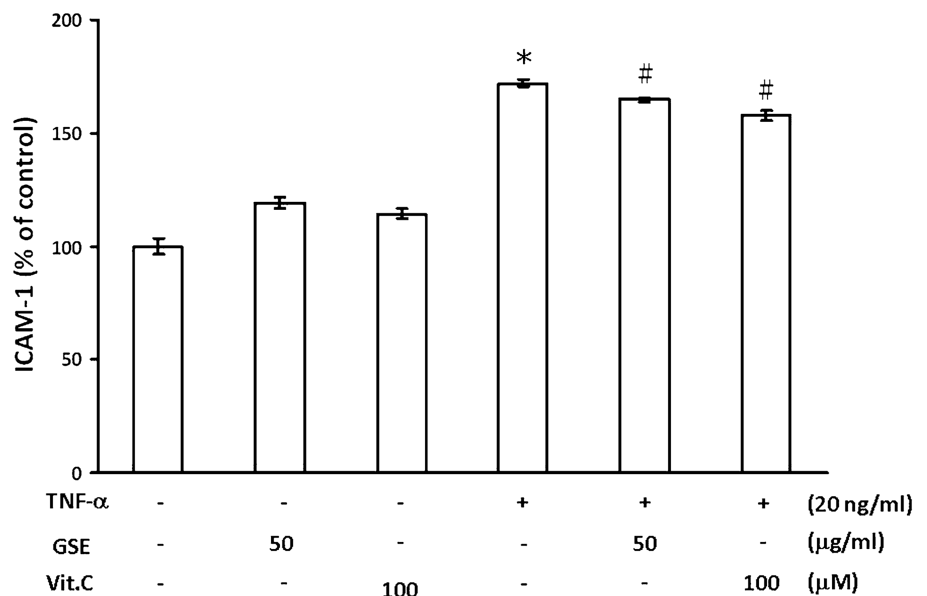

Fig. 5 Effect of GSE and vitamin C on ICAM-1 expression in HUVECs. TNF-α (20 ng/mL) increased the expression of ICAM-1. The TNF-α-induced ICAM-1 expression was reduced by GSE (50 μg/mL) and vitamin C (100 μM). Cells were treated with TNF-α alone or in the presence of GSE or vitamin C for 6 h. The measurement of ICAM-1 was performed by ELISA as described in "Methods". Data are mean ± SEM ($n = 4$). *$P < 0.05$ vs. control, #$P < 0.05$ vs. TNF-α (20 ng/mL)

expression. The inhibitory effect of vitamin C (100 μM) on TNF-α-induced COX-2 expression was also prominent (Fig. 6).

Effect of GSE and vitamin C on PGE_2 production

TNF-α (20 ng/mL) significantly increased PGE_2 production in HUVECs. The TNF-α-induced PGE_2 production was significantly reduced by GSE (50 μg/mL) and vitamin C (100 μM) (Fig. 7).

Discussion

The cardinal findings of this study indicated that in HUVECs, GSE (50–100 μg/mL), a complex mixture containing mainly active ingredients of flavonoids, significantly attenuated TNF-α-induced 8-OHdG production, THP-1 adhesion, the expression of IκB degradation, ICAM-1 and COX-2, and the production of PGE_2.

TNF-α, a potent cytokine that is predominantly produced by macrophages, has been reported to increase the

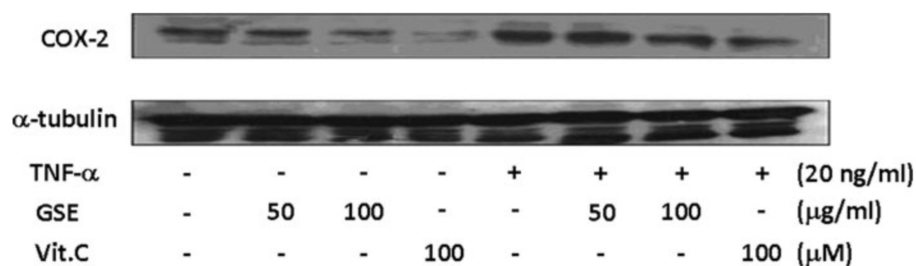

Fig. 6 Effect of GSE and vitamin C on COX-2 expression in HUVECs. The expression of COX-2 was increased by TNF-α (20 ng/mL). GSE showed a dose-dependent manner in inhibiting TNF-α-induced COX-2 expression. Cells were treated with TNF-α alone or in the presence of GSE or vitamin C for 6 h. Western blotting to identify COX-2 was performed as described in "Methods". The data are from a representative experiment of three experiments with similar results

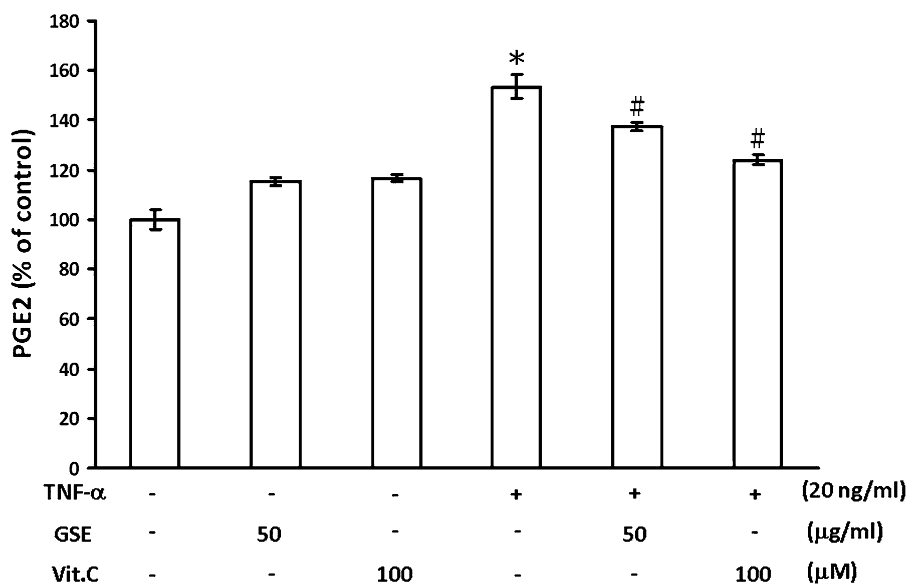

Fig. 7 Effect of GSE and vitamin C on PGE_2 production in HUVECs. TNF-α (20 ng/mL) increased the production of PGE_2. The TNF-α-induced PGE_2 production was reduced by GSE (50 μg/mL) and vitamin C (100 μM). Cells were treated with TNF-α alone or in the presence of GSE or vitamin C for 6 h. The measurement of PGE_2 was performed by ELISA as described in "Methods". Data are mean ± SEM ($n = 4$). $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. TNF-α (20 ng/mL)

production of reactive oxygen species (ROS) such as superoxide anion via NADPH oxidase in endothelial cells, subsequently activating NF-κB to enter the nucleus and induce pro-inflammatory gene expression [22]. Without stimulation, NF-κB is present in the cytoplasm as an inactive complex bound by IκB (an inhibitor of NF-κB); on stimulation, phosphorylation and degradation of IκB occur, and NF-κB gets unmasked and translocated into the nucleus [23]. 8-OHdG has been known as an indicator of oxidative damage to DNA and thought to be related to cellular apoptosis in HUVECs [24]. In the present study, we also demonstrated that 8-OHdG could efficiently evaluate the status of oxidative stress in TNF-α-induced inflammatory process of HUVECs. As to the inhibitory effect of GSE on NF-κB activity, it has been demonstrated in LLC-PK_1 tubule cells [25], but not yet in HUVECs. Using IκB degradation as an indicator of NF-κB activation, we found that the protein level of IκB rapidly degraded after TNF-α treatment; the rapid decrease in IκB expression was similar to that observed in high glucose–induced apoptosis in HUVECs [26].

TNF-α is also thought to cause initiation of atherosclerosis by up-regulating the expression of adhesion molecules on the endothelial cell surface [22]. The leukocyte adhesion includes rolling adhesion (mediated by selectins), firm adhesion (facilitated by VCAM-1 and ICAM-1), and then transmigration through endothelial cell junctions (governed by platelet/endothelial cell adhesion molecule-1), which prompts the progression of atherosclerosis to the next stage [4]. ICAM-1 is much closer to the endothelial junctions and thought to play a more important role than VCAM-1 in transmigration [4]. The in vitro studies conducted to evaluate the regulatory effects of GSE on adhesion molecules were few [27, 28]. One study showed that pretreatment with grape seed proanthocyanidin extract decreased the adherence of Jurkat T cells to TNF-α-treated HUVECs, down-regulated VCAM-1 expression but not ICAM-1 expression [27]. Another study also revealed selective inhibition of advanced glycation end products–induced VCAM-1 by GSPE [28]. In contrast, GSE, being a mixture of polyphenols in our study, effectively decreased ICAM-1 expression. The exact mechanism of the different

results between studies was not clear. The distinctive regulatory pathways of VCAM-1 and ICAM-1 expression might contribute to part of the explanation [29, 30].

Previous study reported that COX-2 expression could be induced in TNF-α-treated HUVECs via activation of p38 MAP kinase [7]. COX-2, via converting arachidonic acid to prostanglandins (PGE_2 and PGI_2), may enhance cell adhesion [7], increase endothelial cell permeability [9], up-regulate monocyte-derived macrophages [31], and lead to plaque rupture [10], although some COX-2 inhibitors were reported to increase the risk of cardiovascular events [32]. Flavonoids, such as kaempferol and quercetin, inhibited COX-2 expression in cytokines-treated HUVECs in a concentration-dependent manner at 5–50 μM [33]. In our study, GSE also showed a dose-dependent effect on the inhibition of COX-2 expression. We did not compare the potency between different flavonoids; however, we provided the comparison results of GSE and vitamin C as a reference for the readers.

Vitamin C is a well-known strong antioxidant and also has anti-inflammatory properties. Although vitamin C therapy failed to show cardiovascular benefits in most of the clinical observational and prospective studies [15], its role as one of the combination agents might be rational [34, 35]. The single concentration of vitamin C (100 μM) chosen for reference in the present study, though above the ranges of physiological concentrations (means around 30–40 μM) [21, 36], was based on the experience of our previous study [37] and the study conducted by Rössig et al. [38], which showed that the concentration of vitamin C (100 μM) effectively inhibited TNF-α (50 ng/mL)-induced apoptosis in HUVECs.

Grape seeds, which consist of lipid, protein, carbohydrates, and 5–8% phenols by weight, contain two-thirds of the extractable phenols of the grapes [18, 39]. The phenols are essentially flavonoids, which are referred to as monomeric flavan-3-ols (molecular weight about 290) [40], with polymerization in the range of 1–20 [41], consisting of about 8% monomers, 70% oligomers (dimers to heptamers), and 22% polymers (above heptamers) [42]. The antioxidant effects of GSE have been extensively studied [18]. In vitro studies showed that GSE reduced oxidized low-density lipoprotein [43] and was an even more potent scavenger of oxygen free radicals when compared with vitamin C and vitamin E [44]. In animal studies, GSE decreased free radical-induced lipid peroxidation in aged rats [45] and attenuated the development of atherosclerosis in cholesterol-fed rabbits [46]. In human studies, GSE reduced postprandial antioxidant levels in smokers [47] and improved flow-mediated dilatation in high-risk subjects [48].

The GSE in our study was a mixture of polyphenols, containing actually one portion of GSE and three portions of citrus flavonoids from sweet oranges (*Citrus sinensis*), the major component of which is hesperidin, a flavanone glycoside with molecular weight about 610 [40]. This commercial product has been analyzed and demonstrated to contain flavonoids and condensed tannins [49]. Like vitamin C (100 μM), the concentrations of GSE in this study was also above the physiological range.

It has been reported that the physiological concentrations of polyphenols are presumed not to exceed 10 μM [14, 50–52]. Therefore, it has been questioned that the physiological concentrations of polyphenols are too low for antioxidative actions [53]. However, the polyphenols in food could be turned into more active metabolites via digestive and hepatic activity after the intestinal absorption. Therefore, the actual total plasma concentration of polyphenols is thought to be substantially higher due to the presence of metabolites, which are usually unable to be detected by present measurements [51, 52].

An example of more powerful effects of active metabolites was that the concentration of quercetin metabolites (sulfate/glucuronide) (1 μM) had comparable antioxidant and anti-apoptotic effects on high glucose–treated HUVECs in comparison with vitamin C (100 μM) and quercetin (the aglycone) (10–50 μM) [37]. Another example was that oral consumption of vitamin C (2 g/d) and GSE (2 g/d) (1 g of polyphenols) could improve flow-mediated vasodilatation in humans [48, 54]. According to these two human studies, taking into account of the comparable power of vascular protection between vitamin C and polyphenols, the estimated plasma concentrations were 75 and 50 μM, respectively [14, 50]. Since the estimated plasma concentration of polyphenols (50 μM) was thought about 10 times higher than the parent polyphenols, it seemed that the active metabolites significantly contribute to the vascular protection [14]. In the present study, we were not able to indicate the exact chemical compositions of GSE that contribute their biological effects. For quality control, further studies are needed to identify the beneficial effects of its specific components.

In conclusion, we demonstrate that GSE effectively ameliorates TNF-α-induced inflammatory status of HUVECs. The findings of the present study suggest that the consumption of GSE may be beneficial to inflammatory atherosclerosis.

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References

1. Libby P (2006) Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr 83:456S–460S
2. Packard RR, Libby P (2008) Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem 54:24–38
3. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R (2008) Atherosclerosis and oxidative stress. Histol Histopathol 23:381–390
4. Wittchen ES (2009) Endothelial signaling in paracellular and transcellular leukocyte transmigration. Front Biosci 14:2522–2545
5. Kobashi C, Urakaze M, Kishida M, Kibayashi E, Kobayashi H, Kihara S, Funahashi T, Takata M, Temaru R, Sato A, Yamazaki K, Nakamura N, Kobayashi M (2005) Adiponectin inhibits endothelial synthesis of interleukin-8. Circ Res 97:1245–1252
6. Lombardi A, Cantini G, Piscitelli E, Gelmini S, Francalanci M, Mello T, Ceni E, Varano G, Forti G, Rotondi M, Galli A, Serio M, Luconi M (2008) A new mechanism involving ERK contributes to rosiglitazone inhibition of tumor necrosis factor-alpha and interferon-gamma inflammatory effects in human endothelial cells. Arterioscler Thromb Vasc Biol 28:718–724
7. Dormond O, Bezzi M, Mariotti A, Ruegg C (2002) Prostaglandin E2 promotes integrin alpha Vbeta 3-dependent endothelial cell adhesion, rac-activation, and spreading through cAMP/PKA-dependent signaling. J Biol Chem 277:45838–45846
8. Mark KS, Trickler WJ, Miller DW (2001) Tumor necrosis factor-alpha induces cyclooxygenase-2 expression and prostaglandin release in brain microvessel endothelial cells. J Pharmacol Exp Ther 297:1051–1058
9. Barbieri SS, Weksler BB (2007) Tobacco smoke cooperates with interleukin-1beta to alter beta-catenin trafficking in vascular endothelium resulting in increased permeability and induction of cyclooxygenase-2 expression in vitro and in vivo. FASEB J 21:1831–1843
10. Cipollone F, Fazia ML, Iezzi A, Cuccurullo C, De Cesare D, Ucchino S, Spigonardo F, Marchetti A, Buttitta F, Paloscia L, Mascellanti M, Cuccurullo F, Mezzetti A (2005) Association between prostaglandin E receptor subtype EP4 overexpression and unstable phenotype in atherosclerotic plaques in human. Arterioscler Thromb Vasc Biol 25:1925–1931
11. Kinlay S, Egido J (2006) Inflammatory biomarkers in stable atherosclerosis. Am J Cardiol 98:2P–8P
12. Wilson PW (2008) Evidence of systemic inflammation and estimation of coronary artery disease risk: a population perspective. Am J Med 121:S15–S20
13. Kaliora AC, Dedoussis GV, Schmidt H (2006) Dietary antioxidants in preventing atherogenesis. Atherosclerosis 187:1–17
14. Ulrich-Merzenich G, Zeitler H, Vetter H, Kraft K (2009) Synergy research: vitamins and secondary plant components in the maintenance of the redox-homeostasis and in cell signaling. Phytomedicine 16:2–16
15. Willcox BJ, Curb JD, Rodriguez BL (2008) Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. Am J Cardiol 101:75D–86D
16. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342:1007–1011
17. Huxley RR, Neil HA (2003) The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. Eur J Clin Nutr 57:904–908
18. Kar P, Laight D, Shaw KM, Cummings MH (2006) Flavonoid-rich grape seed extracts: a new approach in high cardiovascular risk patients? Int J Clin Pract 60:1484–1492
19. Ferrières J (2004) The French paradox: lessons for other countries. Heart 90:107–111
20. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA (2009) Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res 58:537–552
21. Chao CL, Hou YC, Chao PD, Weng CS, Ho FM (2009) The antioxidant effects of quercetin metabolites on the prevention of high glucose-induced apoptosis of human umbilical vein endothelial cells. Br J Nutr 101:1165–1170
22. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, Dellsperger KC, Zhang C (2009) Role of TNF-alpha in vascular dysfunction. Clin Sci (Lond) 116:219–230
23. Csiszar A, Wang M, Lakatta EG, Ungvari Z (2008) Inflammation and endothelial dysfunction during aging: role of NF-kappaB. J Appl Physiol 105:1333–1341
24. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A (2003) Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes 52:2795–2804
25. Yang Y, Yang Y, Xu Y, Lick SD, Awasthi YC, Boor PJ (2008) Endothelial glutathione-S-transferase A4–4 protects against oxidative stress and modulates iNOS expression through NF-kappaB translocation. Toxicol Appl Pharmacol 230:187–196
26. Ho FM, Lin WW, Chen BC, Chao CM, Yang CR, Lin LY, Lai CC, Liu SH, Liau CS (2006) High glucose-induced apoptosis in human vascular endothelial cells is mediated through NF-kappaB and c-Jun NH2-terminal kinase pathway and prevented by PI3 K/Akt/eNOS pathway. Cell Signal 18:391–399
27. Sen CK, Bagchi D (2001) Regulation of inducible adhesion molecule expression in human endothelial cells by grape seed proanthocyanidin extract. Mol Cell Biochem 216:1–7
28. Zhang FL, Gao HQ, Wu JM, Ma YB, You BA, Li BY, Xuan JH (2006) Selective inhibition by grape seed proanthocyanidin extracts of cell adhesion molecule expression induced by advanced glycation end products in endothelial cells. J Cardiovasc Pharmacol 48:47–53
29. Wölle J, Hill RR, Ferguson E, Devall LJ, Trivedi BK, Newton RS, Saxena U (1996) Selective inhibition of tumor necrosis factor-induced vascular cell adhesion molecule-1 gene expression by a novel flavonoid. Lack of effect on transcription factor NF-kappa B. Arterioscler Thromb Vasc Biol 16:1501–1508
30. Chen JW, Chen YH, Lin FY, Chen YL, Lin SJ (2003) Ginkgo biloba extract inhibits tumor necrosis factor-alpha-induced reactive oxygen species generation, transcription factor activation, and cell adhesion molecule expression in human aortic endothelial cells. Arterioscler Thromb Vasc Biol 23:1559–1566
31. Martínez-González J, Badimon L (2007) Mechanisms underlying the cardiovascular effects of COX-inhibition: benefits and risks. Curr Pharm Des 13:2215–2227
32. Cipollone F, Cicolini G, Bucci M (2008) Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives. Pharmacol Ther 118:161–180
33. Crespo I, García-Mediavilla MV, Gutiérrez B, Sánchez-Campos S, Tuñón MJ, González-Gallego J (2008) A comparison of the effects of kaempferol and quercetin on cytokine-induced proinflammatory status of cultured human endothelial cells. Br J Nutr 100:968–976
34. Tousoulis D, Antoniades C, Tentolouris C, Tsioufis C, Toutouza M, Toutouzas P, Stefanadis C (2003) Effects of combined administration of vitamins C and E on reactive hyperemia and inflammatory process in chronic smokers. Atherosclerosis 170:261–267
35. Rizzo MR, Abbatecola AM, Barbieri M, Vietri MT, Cioffi M, Grella R, Molinari A, Forsey R, Powell J, Paolisso G (2008)

Evidence for anti-inflammatory effects of combined administration of vitamin E and C in older persons with impaired fasting glucose: impact on insulin action. J Am Coll Nutr 27:505–511
36. Castilla P, Echarri R, Dávalos A, Cerrato F, Ortega H, Teruel JL, Lucas MF, Gómez-Coronado D, Ortuño J, Lasunción MA (2006) Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. Am J Clin Nutr 84:252–262
37. Wynne H, Khan T, Avery P, Wood P, Ward A, Kamali F (2006) Dietary related plasma vitamin C concentration has no effect on anticoagulation response to warfarin. Thromb Res 118:501–504
38. Rössig L, Hoffmann J, Hugel B, Mallat Z, Haase A, Freyssinet JM, Tedgui A, Aicher A, Zeiher AM, Dimmeler S (2001) Vitamin C inhibits endothelial cell apoptosis in congestive heart failure. Circulation 104:2182–2187
39. Shi J, Yu J, Pohorly JE, Kakuda Y (2003) Polyphenolics in grape seeds- biochemistry and functionality. J Med Food 6:291–299
40. Ross JA, Kasum CM (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 22:19–34
41. Cheynier V (2005) Polyphenols in foods are more complex than often thought. Am J Clin Nutr 81:223S–229S
42. Nair MP, Kandaswami C, Mahajan S, Nair HN, Chawda R, Shanahan T, Schwartz SA (2002) Grape seed extract proanthocyanidins downregulate HIV-1 entry coreceptors, CCR2b, CCR3 and CCR5 gene expression by normal peripheral blood mononuclear cells. Biol Res 35:421–431
43. Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG, Vinson JA (2003) Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. Mutat Res 523–524:87–97
44. Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ (1997) Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. Res Commun Mol Pathol Pharmacol 95:179–189
45. Balu M, Sangeetha P, Haripriya D, Panneerselvam C (2005) Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. Neurosci Lett 383:295–300
46. Yamakoshi J, Kataoka S, Koga T, Ariga T (1999) Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. Atherosclerosis 142:139–149
47. Natella F, Belelli F, Gentili V, Ursini F, Scaccini C (2002) Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. J Agric Food Chem 50:7720–7725
48. Clifton PM (2004) Effect of grape seed extract and quercetin on cardiovascular and endothelial parameters in high-risk subjects. J Biomed Biotechnol 2004:272–278
49. Hsu CP, Lin YH, Chou CC, Zhou SP, Hsu YC, Liu CL, Ku FM, Chung YC (2009) Mechanisms of grape seed procyanidin-induced apoptosis in colorectal carcinoma cells. Anticancer Res 29:283–290
50. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S–2085S
51. Manach C, Williamson G, Morand C, Scalbert A, Remesy C (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
52. Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 81:243S–255S
53. Azzi A (2007) Molecular mechanism of alpha-tocopherol action. Free Radic Biol Med 43:16–21
54. Antoniades C, Tousoulis D, Tountas C, Tentolouris C, Toutouza M, Vasiliadou C, Tsioufis C, Toutouzas P, Stefanadis C (2004) Vascular endothelium and inflammatory process, in patients with combined Type 2 diabetes mellitus and coronary atherosclerosis: the effects of vitamin C. Diabet Med 21:552–558