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An aqueous extract of Welsh onion green leaves increase ABCA1 and SR-BI expression in macrophage RAW 264.7 cells

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Abstract

The effects of the aqueous extracts of Welsh onion green leaves (WOE) on the expression of scavenger receptor class BI (SR-BI) and ATP-binding cassette A1 (ABCA1), the two high-density lipoprotein (HDL) receptors, presented in macrophage RAW 264.7 cells were investigated. WOE in the range of 0–0.5 mg/ml increased the protein expression of ABCA1 and SR-BI by a dose- and time-dependent manner in macrophages. On the other hand, lipopolysaccharide (LPS) at 200 ng/ml decreased ABCA1 and SR-BI protein expression, and WOE in the range of 0–1.0 mg/ml blocked LPS-decreased ABCA1 and SR-BI protein expression. Quercetin and kaempferol, the two major flavonoids presented in WOE, in the range of 0–3.4 μg/ml also showed an inductive effect on the ABCA1 and SR-BI protein and a protective effect under LPS stimulation. Furthermore, determined by reverse transcription-polymerase chain reaction (RT-PCR), WOE in the range of 0–1.0 mg/ml increased the ABCA1 and SR-BI mRNA levels after 12 h treatment. LPS also obviously decreased ABCA1 and SR-BI mRNA in RAW 264.7 cells. These LPS resulted mRNA inhibitions were also markedly prevented by the addition of WOE, quercetin and kaempferol, respectively. These results suggest that WOE and its two major flavonoids, quercetin and kaempferol; have potential effect on increasing HDL receptor expression in macrophages.

Keywords: Welsh onion; Flavonoid; Lipopolysaccharide (LPS); SR-BI; ABCA1

1. Introduction

It is generally accepted that there are two critical steps, including plasma low-density lipoprotein (LDL) modification (Heinecke, 1998) and lipid-laden foam cells formation (Babaev, Patel, Semenkovich, Fazio, & Linton, 2000; Hegyi, Skepper, Cary, & Mitchinson, 1996; Robbesyn, Salvayre, & Negre-Salvayre, 2004), which have far-reaching effects in resulted atherosclerosis lesions. These lipid-laden foam cells, resulting from accumulation of excess intracellular cholesterol in arterial macrophages, play an intensified role in the progression of arterial fatty streaks and atherosclerotic complex lesions (Geng & Libby, 2002; Nicholson, 2004). The final progression of these lesions is tended to make necrotic plaque rupture and this finally induces fatal thrombosis (Viles-Gonzalez, Fuster, & Badimon, 2004). Therefore, the decrease of intracellular cholesterol accumulation in arterial macrophage and inhibition of foam cell formation may be an important goal in atherosclerosis prevention. In light of this, many studies have revealed that high density lipoprotein (HDL) and apolipoprotein A-I can carry cholesterol from lipid-laden macrophages to inhibit foam cell formation (Panagotopulos et al., 2002) and further decrease atherosclerotic lesion progression (Choudhury et al., 2004; Hovingh et al., 2005). In fact, Ji et al. (1997) suggested that scavenger receptor class BI (SR-BI) bind HDL and then mediate aqueous diffusion

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of intracellular cholesterol to HDL, which results in intracellular cholesterol efflux. On the other hand, other researchers noted that ATP-binding cassette (ABC) transporters were presented in macrophages (Langmann et al., 1999; Schmitz et al., 1999) and suggested that the ABCA1 may mediate the efflux of intracellular cholesterol onto lipid-deficient apolipoproteins or HDL (Oram & Vaughan, 2000; Oram, 2003). Unquestionably, ABCA1 and SR-BI have been now recognised as key regulators of HDL-mediated cholesterol efflux and arterial macrophage-derived foam cell formation. However, Feingold et al. (1993) reported that LPS, the structural components of the outer membrane of Gram-negative bacteria, decreased plasma levels of HDL. Baranova et al. (2002) revealed that LPS decreased the expression of SR-BI and ABCA1 mRNA in macrophages. Recalde et al. (2004) also noted that LPS increased the size of atherosclerosis lesions in apolipoprotein E-deficient mice. These data implied that LPS depressing the expression of SR-BI and ABCA1 was a factor in atherosclerogenesis progression.

Dietary antioxidants (e.g. polyphenol and flavonoid) have been shown to play some protective effects on LDL oxidation (Fuhrman & Aviram, 2001) and atherosclerosis progression (Gottstein et al., 2003). Meanwhile, increasing dietary flavonoid intake has been reported to be inversely associated with the evidence of coronary artery disease (Tucker, 2004). Therefore, the capacity of these dietary flavonoid antioxidants to reduce atherogenesis and coronary artery disease might be correlated to their induction in SR-BI and ABCA1 protein expression resulting in reducing cholesterol accumulation in the arterial wall. In addition, Welsh onion (*Allium fistulosum* L., Alliaceae) has been suggested to lower blood pressure (Chen et al., 2000), modulate aortic vascular tone (Chen, Tsai, & Chen, 1999) and inhibit platelet aggregation (Chen et al., 2000). Other studies have indicated that garlic and onion, members of the *Allium* family, may be used as anti-atherosclerosis (Campbell, Efendy, Smith, & Campbell, 2001), anti-thrombotic (Jung et al., 2002) and anti-hyperlipemic agents (Yeh & Liu, 2001). In our previous studies (Wang, Chen, Liang, & Duh, 2005), WOE inhibited copper-induced LDL oxidation and LPS-induced nitric oxide production in macrophages. Although these biological functions of Welsh onion have been demonstrated to be beneficial to the coronary artery disease prevention, whether WOE could affect SR-BI and ABCA1 expression in macrophages has not been elucidated so far. Furthermore, quercetin and kaempferol have been identified as the principal flavonoid constituents in WOE (Chen & Tsai, 1999). Whether these dietary flavonoid constituents could affect SR-BI and ABCA1 is still unclear. Thus, the aim of this study was to explore the effect of WOE, quercetin and kaempferol on the expression of ABCA1 and SR-BI in macrophages, with and without the presence of LPS. Results suggest that WOE and its two major flavonoid constituents, quercetin and kaempferol, have potential effect on increasing ABCA1 and SR-BI expression in macrophages.

2. Materials and method

2.1. Materials

Fresh mature Welsh onions were obtained from 10 local markets in Tainan, Taiwan. The batches of Welsh onion were mixed well, and then used for WOE preparation. Lipopolysaccharide (LPS, *Escherichia coli* 0127:B8), quercetin and kaempferol were purchased from Sigma (St. Louis, MO, USA). TRIzol reagent was obtained from Life Technologies (California, USA).

2.2. Preparation of aqueous extract of Welsh onion green leaves (WOE)

The green leaves portion (1000 g) of the Welsh onion was squeezed and filtered by a food processor (National, Model MJ-C85; Tokyo, Japan; pore size of the filter: 0.2 mm) to obtain the Welsh onion green leave juice (400 g). After centrifugation at 10,000*g* for 30 min, the supernatant (340 g) was the aqueous extract of Welsh onion green leave (WOE). Furthermore, the extract was filtered, frozen at −80 °C, and then lyophilised for 24 h. This dehydrated powder (20 g) was then dissolved in phosphate buffer saline for each assay (Wang et al., 2005).

2.3. Cell culture

RAW 264.7 cells, an Abelson virus-transformed murine macrophage cell line (American Type Culture Collection), was cultured in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM pyruvate and were maintained in humidified 5% CO_2/95% air at 37 °C. For immunoblot and RT-PCR analysis, cells were grown in a dish with growth medium for 18 h to allow the cell number to approximately double. Then, the growth medium was replaced and WOE was added with or without the presence of LPS.

2.4. Western blot

After stimulation, cells were washed with ice-cold phosphate buffer saline, and then cells were treated with lysis buffer. Cellular lysates were centrifuged at 10,000*g* at 4 °C for 20 min. The supernatants were collected and the protein contents were determined by using the BCA protein assay kit (Piece, USA). Each sample, which contained 50 μg proteins, was separated on 8% SDS-polyacrylamide gels. After electrophoresis, gels were transferred to nitrocellulose paper. After washing with distilled water, the membrane was incubated with 5% albumin in phosphate buffer saline (containing 0.1% Tween-20) and then immunoblotted with rabbit polyclonal anti-SR-BI antibody (CALBIOCHEM, Germany), rabbit polyclonal anti-ABCA1 antibody (NOVUS, USA) or mouse monoclonal anti-Tubulin antibody (Sigma, USA). Blots were then incubated with secondary IgG antibody conjugated to horseradish

peroxidase (Santa Cruz, USA) and visualised using an enhanced chemiluminescence (ECL) kit (Amersham, USA) (Wang et al., 2005). The expression levels of ABCA1, SR-BI and Tubulin proteins were determined by densitometry and analysed.

2.5. Reverse transcripion-polymerase chain reaction (RT-PCR)

Total RNA was extracted from RAW 264.7 cells, collected after WOE and LPS treatment, using TRIzol Reagent (Life technologies, USA) according to the manufacturer's instructions. Then, RNA concentrations were determined spectrophotometrically and 5 μg RNA was converted to cDNA with 1 μM oligo (dT) 15, 500 μM of each dNTP, 50 mM Tris–HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, RNase inhibitor (1 unit/μl) and moloney-murine leukemia virus reverse transcriptase (10 unit/μl) at 42 °C for 1 h. One μl of the resulting cDNA was used for amplification, by specific primers for SR-BI, ABCA1 and GAPDH. The amplification of cDNA was carried out by incubating in 0.1 M Tris–HCl buffer, pH 8.3 containing 0.5 M KCl, 0.015 M $MgCl_2$, 0.1% gelatin, 0.2 mM dNTPs and 50 unit/ml of Super Taq DNA polymerase with various primers. Number of cycles, temperatures and primers for mouse SR-BI, ABCA1 and GAPDH were as previously described (Baranova et al., 2002; Wang et al., 2005). PCR products were analysed on 1.8% agarose gels. Amplified cDNA bands were detected by ethidium bromide staining and quantified by densitometry.

2.6. Statistical analysis

The StatView statistical package (SAS institute inc.) was used to analyse data and statistical significance between the groups was assessed by using one-way analysis of variance (ANOVA) followed by comparing all columns with a post hoc test with Bonferroni correction. The level of significance was chosen as $p < 0.05$.

3. Results

3.1. Effects of WOE on ABCA1 and SR-BI protein in RAW 264.7 cells

Fig. 1 shows the effect of WOE on ABCA1 and SR-BI protein expression in RAW 264.7 cell. In the range of 0–0.5 mg/ml, WOE showed the forwarding effect on the ABCA1 protein with a concentration dependent manner after 24 h treatment (Fig. 1a). As shown in Fig. 1b, WOE at 0.2 and 0.5 mg/ml increased the ABCA1 by 176% and 222%, respectively, greater than that of control group. Meanwhile, WOE at 1.0 mg/ml also advanced the level of ABCA1 in a time dependent manner in 24 h (data not shown). In fact, the ABCA1 could be increased by 96% greater than that of the control by 1.0 mg/ml of WOE after a 6 h treatment. On the other hand, the effect of WOE on SR-BI protein was also shown in Fig. 1a. When cells were incubated with WOE for 24 h, WOE (0–0.5 mg/ml) also increased cellular SR-BI protein levels in a dose-dependent manner. When cells were exposed to WOE at 0.2, and 0.5 mg/ml, the protein levels of SR-BI were increased by 35% and 88%, respectively, greater than that of control group in macrophages (Fig. 1b). The induction capacities of WOE on SR-BI protein were inferior to that obtained for ABCA1 protein. These data indicated that WOE exhibited remarkable effects on ABCA1 and SR-BI protein in macrophages.

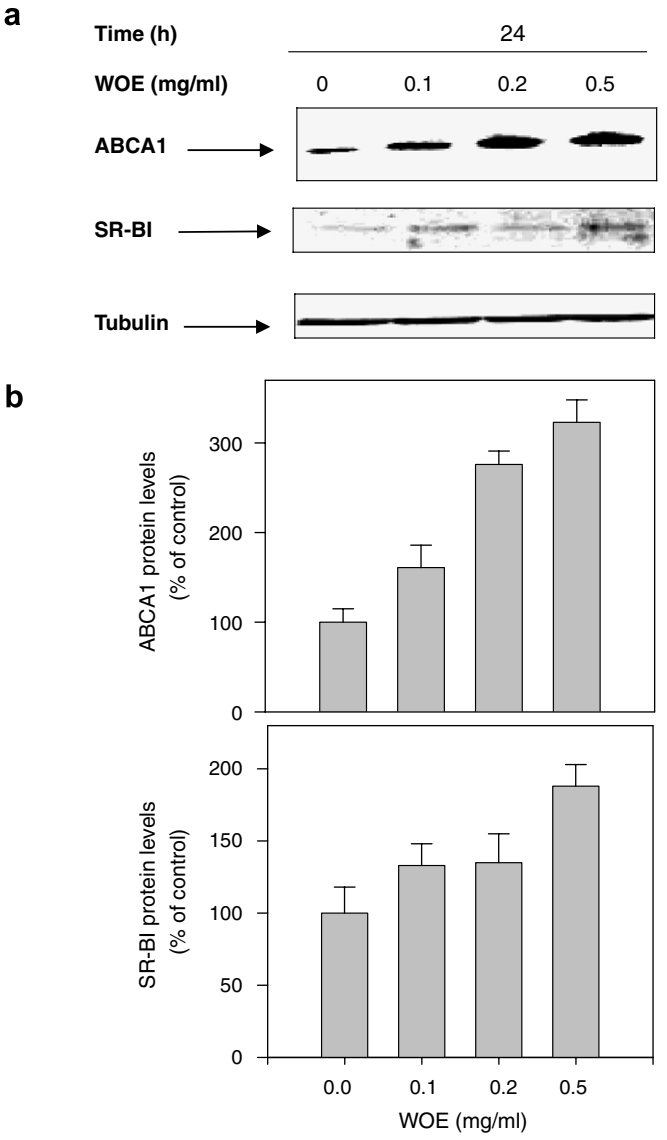

Fig. 1. Effects of aqueous extracts of Welsh onion green leaves (WOE) on (a) ABCA1 and SR-BI protein levels in RAW 264.7 cells. After 24 h treatment, cellular lysates were collected and separated by 8% SDS-polyacrylamide gels and then immunoblotted as described in Section 2. One representative blot of three independent experiments is shown and (b) The densities of ABCA1 and SR-BI were normalised using Tubulin intensities. The data were displayed with mean ± S.D. ($n = 3$) and analysed by ANOVA ($p < 0.05$).

3.2. Effect of WOE on LPS-decreased ABCA1 and SR-BI proteins in RAW 264.7 cells

In order to elucidate the availability of WOE on suppression of LPS in ABCA1 and SR-BI protein expression in macrophages, the effect of WOE on ABCA1 and SR-BI protein expression in the presence of LPS was determined. The efficacy of LPS on ABCA1 and SR-BI production in macrophage was shown in Fig. 2a. When LPS (200 ng/ml) was added to RAW 264.7 cells, ABCA1 and SR-BI proteins were dramatically decreased down to 45% and 38%, respectively, of the basal level of control cells (Fig. 2b). However, the ABCA1 and SR-BI proteins production from LPS-stimulated RAW 264.7 cells were recovered in the presence of WOE (0.5–1.0 mg/ml) (Fig. 2a). When cells were incubated with WOE at 0.5 and 1.0 mg/ml in the presence of LPS for 24 h, the protein levels of ABCA1 protein were increased by 53% and 63%, respectively, greater than the LPS group (Fig. 2b). The protein levels of SR-BI were increased by 116% and 139%, respectively, greater than the LPS group after cells were incubated with WOE at 0.5 and 1.0 mg/ml in the presence of LPS for 24 h in macrophages (Fig. 2b). More increasing capacities of WOE on SR-BI protein than that were obtained for ABCA1 protein in LPS stimulated macrophages. This data demonstrated that WOE could protect cells against depressions of ABCA1 and SR-BI protein in LPS stimulated macrophages.

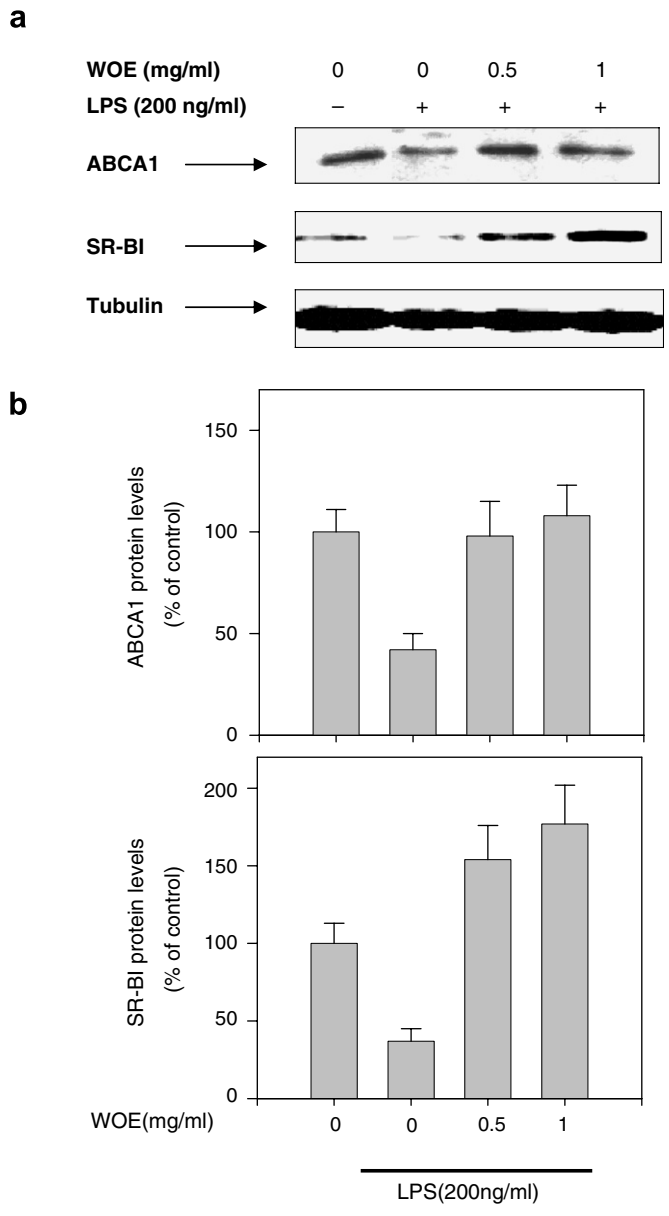

Fig. 2. Effects of aqueous extracts of Welsh onion green leaves (WOE) on LPS-decreased ABCA1 and SR-BI protein levels in RAW 264.7 cells. Cellular lysates were collected after 24 h treatment and separated by SDS-polyacrylamide gels and then immunoblotted: (a) one representative blot of four independent experiments is shown and (b) bars represent mean ± S.D. ($n = 4$) and analysed by ANOVA ($p < 0.05$).

3.3. Effect of WOE on LPS-decreased ABCA1 and SR-BI mRNAs in RAW 264.7 cells

To explore the mechanism responsible for enhancing of ABCA1 and SR-BI production by WOE, whether WOE could directly affect ABCA1 and SR-BI mRNA expression in macrophages was determined. Fig. 3a showed the effect of WOE on ABCA1 and SR-BI mRNA production in RAW 264.7 cells. When cells were treated with WOE in the range of 0–1 mg/ml for 12 h, the ABCA1 and SR-BI mRNA expression were obviously increased (Fig. 3a). According to the results shown in Fig. 3b, when cells were exposed to 0.5 and 1 mg/ml of WOE for 12 h, the mRNA levels of ABCA1 were increased by 41% and 78%, respectively, greater than the control group in macrophages. Further, the increases of SR-BI mRNA levels were increased by 49% and 88%, respectively, greater than the control group in cells in the presence of 0.5 and 1 mg/ml WOE (Fig. 3b). The forwarding activity of WOE on ABCA1 and SR-BI mRNA expression did not increase with higher concentration used (data not shown), WOE did show an increasing effect on ABCA1 and SR-BI mRNA in macrophages. However, as expected, when cells were treated with LPS (200 ng/ml) for 12 h, the ABCA1 and SR-BI mRNA production were clearly decreased by 56% and 57%, respectively, lower than that of control group in macrophages (Fig. 3c and d). Consistent with the results mentioned above, treatment of cells with LPS in the presence of 1.0 mg/ml WOE, the ABCA1 and SR-BI mRNA were increased by 72% and 65%, respectively, greater than the LPS alone group (Fig. 3c and d). These results implied WOE could block the LPS-decreased in both ABCA1 and SR-BI mRNA expression in macrophages.

3.4. Effect of quercetin and kaempferol on LPS-decreased ABCA1 and SR-BI proteins in RAW 264.7 cells

To further verify if the flavonoid components present in WOE, were involved in regulating the ABCA1 and SR-BI production, quercetin and kaempferol, two identified flavonoids present in WOE, were used to culture with cells.

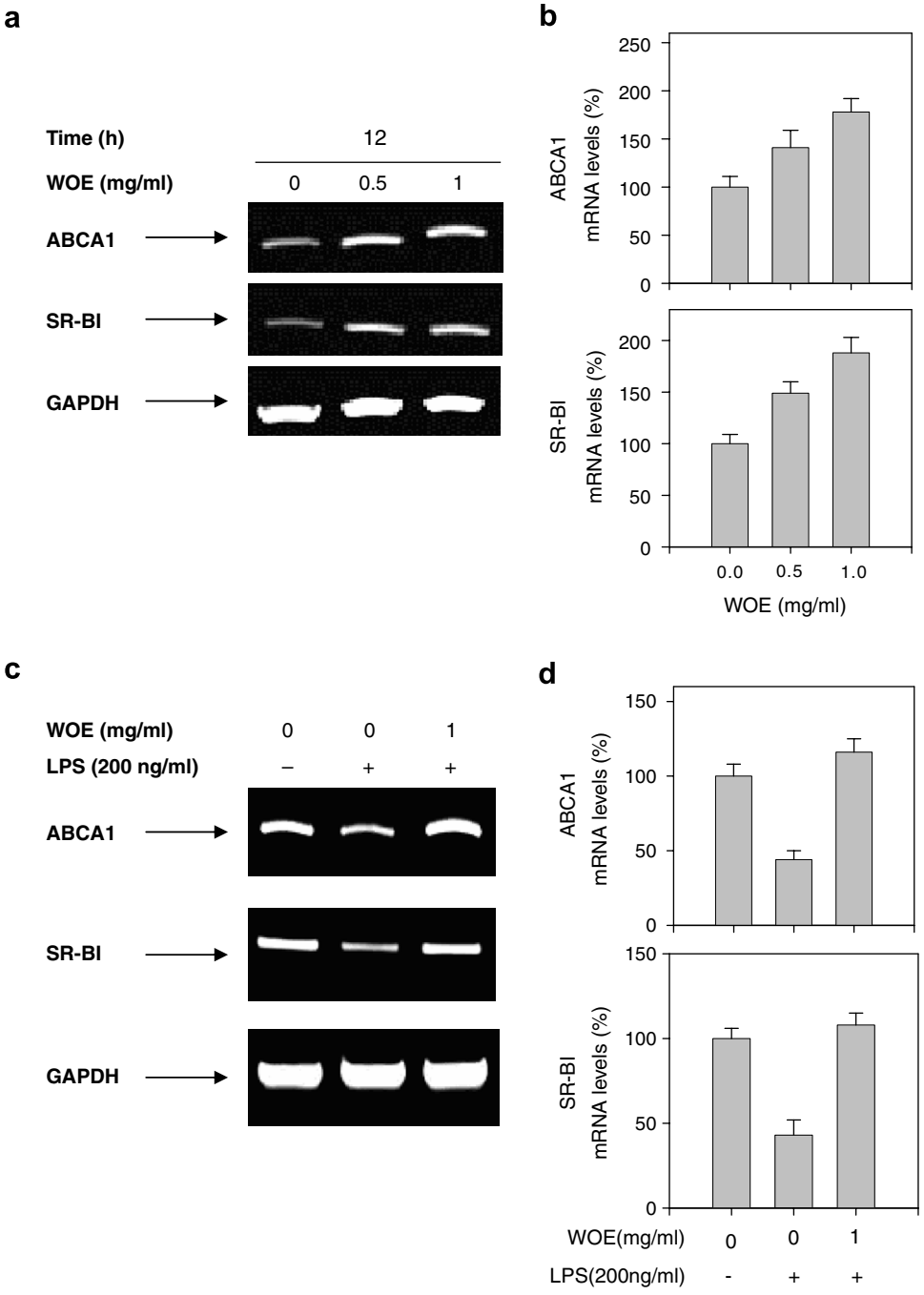

Fig. 3. Effect of aqueous extracts of Welsh onion green leaves (WOE) on the mRNA levels of ABCA1 and SR-BI in RAW 264.7 cells cultured (a and b) without or (c and d) with LPS, (a and c) after 12 h treatment, the total RNA were extracted from cells and first reverse-transcribed. Then, cDNA fragments were amplified by PCR reaction with primers for ABCA1, SR-BI and GAPDH. One representative result of three independent experiments is shown, (b and d) the densities of ABCA1 and SR-BI detected per lane were normalised using GAPDH intensities. The bar data were displayed with mean ± S.D. ($n = 3$) and analysed by ANOVA ($p < 0.05$).

Fig. 4a showed quercetin (Que) and kaempferol (Kae), respectively, increased ABCA1 and SR-BI protein in macrophages. The cells cultured with quercetin (Que) and kaempferol (Kae) in the range of 0–3.4 μg/ml resulted in an obviously induction of ABCA1 and SR-BI protein. Further, as shown in Fig. 4b, in the presence of 3.4 μg/ml quercetin, the ABCA1 and SR-BI protein production were increased by 95% and 65%, respectively, greater than the control group. In the presence of 2.9 μg/ml kaempferol, the ABCA1 and SR-BI protein production were increased by 98% and 75%, respectively, greater than the control group. These results implied that quercetin and kaempferol could increase both ABCA1 and SR-BI protein expression in macrophages.

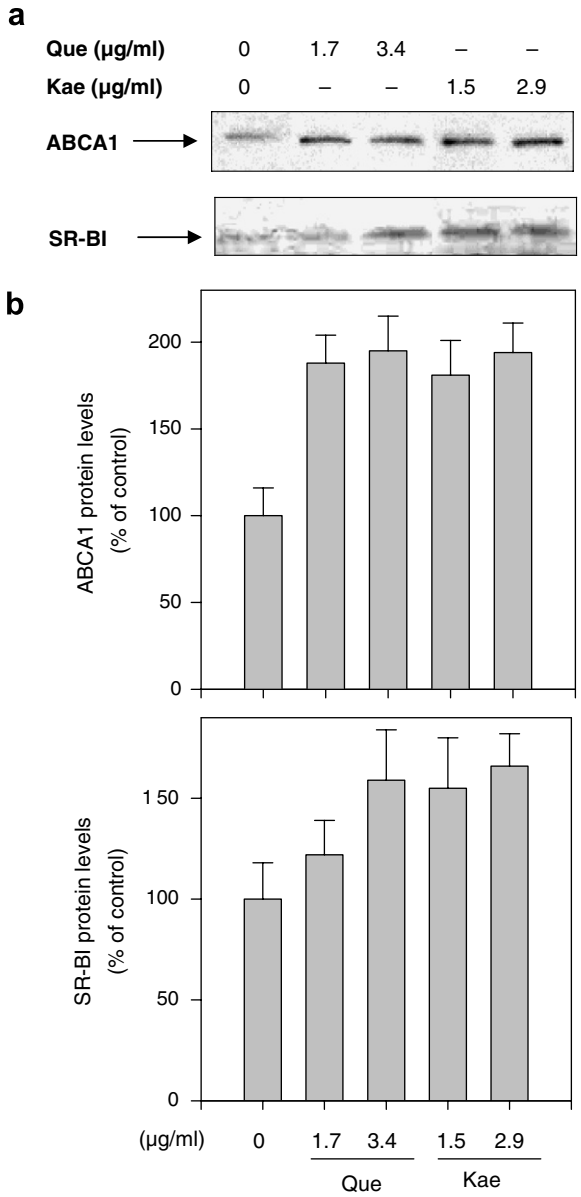

Fig. 4. Effect of quercetin (Que) and kaempferol (Kae) on the ABCA1 and SR-BI protein expression in RAW 264.7 cells: (a) cellular lysates were collected after 24 h treatment and separated by SDS-polyacrylamide gels and then immunoblotted. One representative blot of four independent experiments is shown. The amount of ABCA1 and SR-BI detected per lane were normalised using Tubulin intensities and (b) bars represent means ± S.D. ($n = 4$) and analysed by ANOVA ($p < 0.05$).

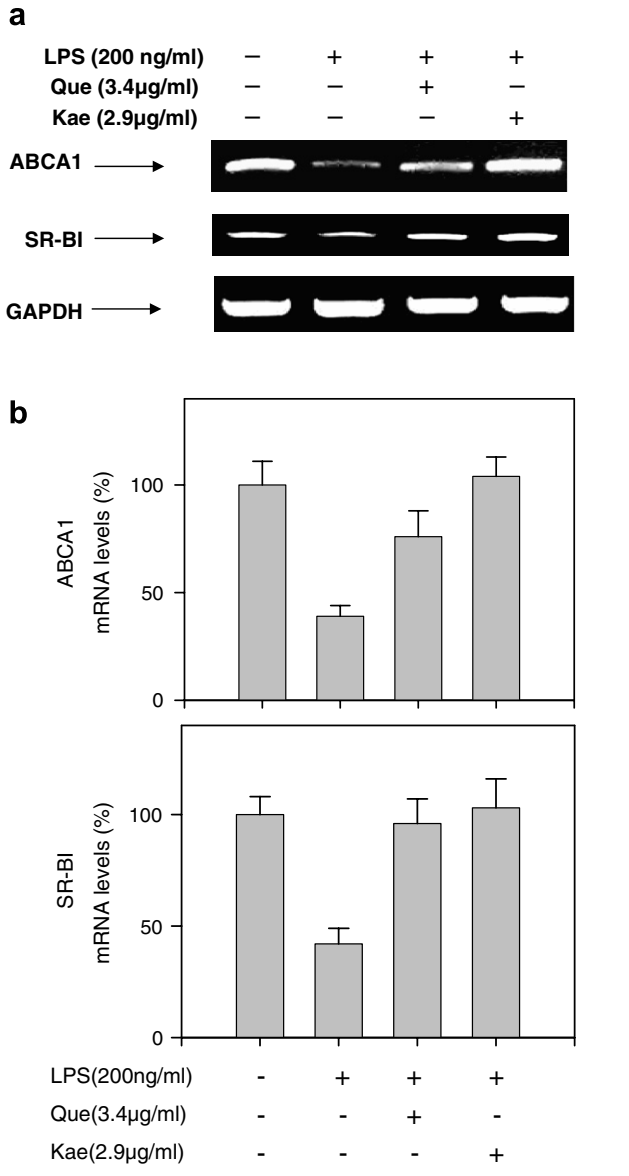

Fig. 5. Effect of quercetin (Que) and kaempferol (Kae) on the mRNA levels of ABCA1 and SR-BI in RAW 264.7 cells cultured with the presence of LPS or not: (a) after 12 h treatment, the total RNA were extracted from cells and first reverse-transcribed. Then, cDNA fragments were amplified by PCR reaction. One representative result of three independent experiments is shown and (b) the densities of ABCA1 and SR-BI detected per lane were normalised using GAPDH intensities. Bars represent means ± S.D. ($n = 3$) and analysed by ANOVA ($p < 0.05$).

3.5. Effect of quercetin and kaempferol on LPS-decreased ABCA1 and SR-BI mRNA in RAW 264.7 cells

To gain further insight of whether quercetin and kaempferol could influence the LPS-decreased ABCA1 and SR-BI mRNA levels in RAW 264.7 cells, cells were treated with quercetin and kaempferol in combination with LPS to determine their ABCA1 and SR-BI mRNA expression. Fig. 5a showed quercetin (Que) and kaempferol (Kae) blocked the ABCA1 and SR-BI mRNA decrease in macrophages treated with LPS. According to the result presented in Fig. 5b, in the presence of LPS (200 ng/ml), the addition of 3.4 μg/ml quercetin to the LPS-stimulated cells could significantly increase ABCA1 and SR-BI mRNA by 37% and 54%, respectively, greater than the LPS alone group. Similarly, addition of 2.9 μg/ml kaempferol to the LPS-stimulated cells also could obviously increase ABCA1 and SR-BI mRNA levels by 65% and 61%, respectively, greater than the LPS group. These results implied quercetin and kaempferol could block the LPS-decreased both ABCA1 and SR-BI mRNA expression in macrophages.

4. Discussion

Fig. 1 showed the increasing effect of WOE on SR-BI and ABCA1 protein in the macrophage RAW 264.7 cell. SR-BI has been known to express abundantly in various tissues (Azhar, Leers-Sucheta, & Reaven, 2003). By formation of a complex with lipoprotein, SR-BI could promote selective uptake of HDL cholesterol in steroidogenic tissues and facilitate the cellular cholesterol efflux to phospholipids containing acceptors to eliminate cholesterol excess in the arterial wall (Francone, 2003). In other words, SR-BI might play a role with holding bi-directional flux of cellular cholesterol processes in the development of atherosclerosis (Temel et al., 1997; Xu et al., 1997). However, Covey, Krieger, Wang, Penman, and Trigatti (2003) have used bone marrow transplantation to selectively eliminate SR-BI expression in bone marrow-derived cells in a hyper-lipidemic mouse model and demonstrated that the absence of SR-BI is associated with increases in atherosclerosis progression. Braun et al. (2002) suggested that loss of SR-BI expression leads to the early onset of atherosclerotic coronary artery disease and spontaneous myocardial infarctions in apolipoprotein E-deficient mice. According to these hypotheses, atherosclerosis would be developed when SR-BI expression was inhibited. The fact that WOE increased the SR-BI protein expression in macrophage could be beneficial to these crucial steps which mediating the removal of excess cholesterol from foam cells in the arterial wall to HDL.

On the other hand, ABCA1 proteins also seem to be expected to serve as an anti-atherogenic role. Unlike SR-BI mediating a bi-directional flux of cholesterol, ABCA1 has been certified to mediate a unidirectional and net efflux of cellular cholesterol across the plasma membrane to lipid-poor apolipoprotein (Oram & Vaughan, 2000; Oram, 2003). van Dam et al. (2002) noted that a clear association between increased human arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux was found. Aiello et al. (2002) noted that inactivation of ABCA1 in macrophages could increase atherosclerosis development in hyper-lipidemic mice. On the contrary, Joyce et al. (2002) reported that overexpression of macrophage ABCA1 proteins in C57Bl/6 mice on a pro-atherogenic diet obviously decreased aortic atherosclerosis. Except for promoting the cellular efflux of cholesterol, the ABCA1 protein decreased cholesterol accumulation in macrophages and reduced the foam cell formation. These increased ABCA1 proteins also leads to increase biliary cholesterol excretion in transgenic mice (Vaisman et al., 2001) and lower the atherogenic apoB-containing lipoproteins (Joyce, Freeman, Brewer, & Santamarina-Fojo, 2003). These studies implied that high levels of SR-BI and ABCA1 expression exhibit large attribution to intracellular cholesterol defluxion and HDL-mediating cholesterol depletion. On these basis, WOE increasing SR-BI and ABCA1 expression could play an anti-atherogenic role.

In fact, decreased oxidative modification of LDL has been considered to be an initial step in inhibition progression of atherosclerosis and cardiovascular diseases (Heinecke, 1998). Wang et al. (2005) had clearly shown that WOE exhibited a significant inhibitory effect on LDL oxidation. The vegetables of the *Allium* species are well known to exhibit significant protection against LDL oxidation. For example, fresh garlic or garlic extracts have beneficial effects on blood lipid and LDL oxidation (Lau, 2001). According to the data in Fig. 1 and the fact that WOE inhibited LDL oxidation (Wang et al., 2005), WOE might play multiple anti-atherogenic roles as a result of inhibiting LDL oxidation and increasing SR-BI and ABCA1 expression in macrophages.

Recent research has found that inflammation plays an essential role in all phases of atherosclerosis formation from the creation of plaques to their growth and rupture (Rohde & Lee, 2003). Stoll et al. (2004) noted bacterial endotoxin (LPS) is a potential source of vascular inflammation and may be an important risk factor for atherosclerosis. Under LPS stimulation, multiple reactive oxygen species (ROS) and reactive nitrogen species (RNS) could be produced from neutrophils and macrophages (Forman & Torres, 2001). These reactive species might oxidise protein and lipid moieties of LDL. However, some studies have reported that RNS (e.g. nitric oxide) are generated during the initiation of atherosclerosis, particularly by macrophages (Alfon, Guasch, Berrozpe, & Badimon, 1999; Aliev et al., 2001). LPS also could promote atheroma development by increasing macrophage-derived foam cell formation (Engelmann, Redl, & Nikol, 2004; Funk, Feingold, Moser, & Grunfeld, 1993). These reports agree that LPS might be a risk factor for arterial inflammation and atherosclerosis development. In previous studies (Wang et al., 2005), WOE inhibited LPS-induced NO production in macrophages. The inhibition on NO production in macrophages by WOE was by blocking NF-κB activation and iNOS expression. In other words, WOE may act as a NF-κB inhibitor. Baranova et al. (2002) had noted that the inhibitors of NF-κB activation played a role in blocking the suppressive effect of LPS on SR-BI and ABCA1. Taking these results together, WOE could inhibit NF-κB activation and then block the suppressive effect of LPS on SR-BI and ABCA1 in macrophages. Consequently, these protective effects might contribute to the well known anti-atherogenic function of Welsh onion in the diet.

According to the data presented above, WOE obviously increased the SR-BI and ABCA1 protein expression, however, the cellular mechanism of WOE for up-regulating the SR-BI and ABCA1 proteins in RAW 264.7 cells is further investigated. Post-transcriptional control of SR-BI expression had been originally suggested by the initial characterisation of SR-BI expression pattern in different tissues. Witt, Kolleck, Fechner, Sinha, and Rustow (2000) have showed that feeding rats with a vitamin E-depleted diet resulted in a 11-fold increase in the SR-BI protein in rat liver, but the level of SR-BI mRNA in the liver did not

change. Mardones et al. (2003) also noted that feeding mice with fibrate-containing chow diet suppressed SR-BI protein expression in the liver without changing steady state SR-BI mRNA levels. Also, Wellington et al. (2002) implied that in certain cells and tissues there is notable discordance between ABCA1 mRNA and protein expression, suggesting that regulation of ABCA1 expression may occur at both transcriptional and post-transcriptional levels. These reports supported that existence of different regulatory mechanism between ABCA1 and SR-BI mRNA and protein expression. As seen in Figs. 1 and 3, WOE displayed different rising effects on the SR-BI and ABCA1 mRNA and protein expression. These data implied both transcriptional and post-transcriptional regulation might contribute to WOE's increasing effects on SR-BI and ABCA1 expression.

In addition, Miean and Mohamed (2001) have reported that the abundant flavonoid compounds were found in onion leaves. Furthermore, Chen and Tsai (1999) have analysed flavonoid compounds present in WOE. Quercetin and kaempferol were identified as the major flavonoid constituents presented in WOE. Quercetin had been reported to attenuate aortic fatty streak accumulation in hypercholesterolemic hamster (Auger et al., 2005). Liang et al. (1999) noted that kaempferol was a potential inhibitor of NF-κB induction at the level of gene transcription. Cho et al. (2003) also reported that quercetin down regulated degradation of IκB-α and NF-κB activation. These data suggested that quercetin and kaempferol might act as an anti-atherogenic role by inhibiting NF-κB activation in inflammatory resulted atheroma development. In Figs. 4 and 5, quercetin and kaempferol showed an obviously potential effect in SR-BI and ABCA1 expression in macrophages. As previously noted, these findings implied that WOE containing quercetin and kaempferol might effectively prevent LPS resulted in SR-BI and ABCA1 depression during acute inflammation by suppressing NF-κB-mediated response. On the other hand, some organosulfur compounds and polysulfides presented in Welsh onion might affect biofunctional properties (Kuo, Chien, & Ho, 1990). Chen and Tsai (1999) had found 1-propenyl propyl thiosulfinate presented in WOE. S-allyl cystein and diallyl disulfide in garlic have also been proved to inhibit LDL oxidation and suppress the formation of atherogenic lesions (Campbell et al., 2001). Whether these organosulfur compounds exhibit a modulator on SR-BI and ABCA1 expression or not merits further study.

In conclusion, WOE increased both SR-BI and ABCA1 protein expression in LPS-stimulated macrophages. With these biological functions, WOE could possess the effects of anti-cholesterol accumulation activity in vascular walls and prevent progress of atherosclerosis under inflammatory stress. Further investigation of the mechanisms of WOE underlying SR-BI and ABCA1 expression regulation might be helpful in prevention of patients with different cardiovascular lesions.

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