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ORIGINAL CONTRIBUTION

Lihui Wang
Chengyan Geng
Liping Jiang
Dezheng Gong
Dayu Liu
Hiroyuki Yoshimura
Laifu Zhong

The anti-atherosclerotic effect of olive leaf extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis

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L. Wang
Dept. of Pathology
Dalian Medical University
Dalian, China

C. Geng · L. Jiang · D. Gong · L. Zhong (✉)
China-Japanese Joint Institute for Medical and Pharmaceutical Science
Dalian Medical University
116044 Dailan, China
Tel./Fax: +86-411/8611-0285
E-Mail: rdrczhong@dlmedu.edu.cn

D. Liu
Biomedical Engineering Center
Improve Medical Instrument's Co. Ltd.
Guangzhou, China

H. Yoshimura
Eisai Food and Chemical Co., Ltd
Tokyo, Japan

Abstract *Background* The anti-atherogenic effect of olive leaf extract is supposed to be related to its activities of anti-oxidation and anti-inflammation. *Aim of the study* To prove the effect of anti-atherosclerosis by olive leaf extract (OLE) and to elucidate the mechanism behind. *Methods* Twenty-four rabbits were assigned to the control, high lipid diet (HLD) and OLE group that were fed with standard diet, HLD and HLD supplemented with OLE, respectively. Serum levels of atherosclerosis related markers, triglyceride (TG), total cholesterol (T-CHO), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and malondialdehyde (MDA) were detected at the ends of week 2, 4 and 6. Surface lesions and thickness of intimas were measured at the end of week6. The protein and/or mRNA expressions of inflammation factors, monocyte chemoattractant protein (MCP)-1, vascular cell adhesion molecule (VCAM)-1, nuclear factor-kappa B (NF-κB) and tumor necrosis factor α (TNF-α) were investigated by immunohistochemistry and RT-PCR. *Results* Atherosclerotic lesions were found in the HLD and OLE groups but not in the control group. In comparison with that in the HLD group, reduced size and thickness of intima (0.31 ± 0.26 in the HLD group versus 0.10 ± 0.03 mm in the OLE group) were found in the OLE group. The MDA level, an indicator of antioxidant status, was 35.27 ± 15.37 in the HLD group and 20.63 ± 11.52 nmol/ml in the OLE group. The level of CHO, TG and LDL-C were 104.46 ± 30.34, 2.48 ± 1.11, 82.83 ± 28.44 mmol/l in the HLD group versus 83.03 ± 27.23, 1.84 ± 0.44, 59.51 ± 23.72 mmol/l in the OLE group. Down-regulated expressions of MCP-1, VCAM-1, NF-κB and TNF-α at both protein and mRNA level ($P < 0.05$) were also found with the administration of OLE. *Conclusion* This study proved the effect of OLE on inhibition of atherosclerosis, which is related to the suppressed inflammatory response.

Key words altherosclerosis – olive leaf extract – inflammatory response – inflammatory factor

Introduction

There is general agreement that the Mediterranean diet, in which olive extracts are important components, contributes to the reduced incidence of cardiovascular diseases [16]. Olive extracts, including olive leaf extract (OLE) and olive oil, have been associated with the effect of anti-atherogenesis. The decreased serum lipid level could be the most important factor

that responsible for the anti-atherosclerotic effect of olive extracts [1, 10]. Several lines of evidence support the finding that olive extracts were strong antioxidants with the activities of anti-oxidation and anti-inflammation, including olive extracts suppress generation of free radical and oxidation of low density lipoprotein (LDL) [35], scavenge reactive oxygen species (ROS) [14], reduce oxidative stress induced by smoking [36], and inhibit aggregation of platelet [17], adhesion of monocyte to stimulated endothelium [7] and carrageenin-induced arthritis in rat [27]. These findings indicate that the inhibition of oxidation and inflammatory response could be a mechanism in addition to the decrease of blood lipid that relate to the anti-atherosclerosis effect of olive extracts.

The pathogenesis of atherosclerosis involves an important inflammatory component especially at the early stage named fatty streak, in which multiple leukocytes and inflammatory factors are involved. Fatty streak consists of lipid-laden macrophages, namely foam cells that derived primarily from recruited monocytes [31]. The recruitment and transendothelial migration of the circulating leukocytes are governed by chemotactic factors. The adhesion of recruited leukocytes, particularly monocytes to the vascular endothelium at sites of injury is mediated by adhesion molecules. The engulfment of lipids and transformation of monocytes into foam cells are mediated by inflammatory factors such as tumor necrosis factor α (TNF-α) [30]. The regulation of adhesion molecules and chemotactic factors are mediated by the redox-sensitive transcription factor nuclear factor-kappa B (NF-κB), which is activated by a multitude of stimuli, including oxidized lipoproteins and cytokines such as TNF-α. Activated NF-κB in turn regulates a series of genes implicated in atherogenesis [4]. The interactions of inflammatory factors contribute to atherogenesis by mediating inflammatory response. OLE are supposed to be involved in the regulations of inflammatory and proinflammatory responses, including proinflammatory gene expression.

The transcription of proinflammatory genes appears to be activated by oxidation but suppressed by antioxidants. The antioxidants suppressed cytokine induced NF-κB activation and expression of vascular cell adhesion molecule-1(VCAM-1) in cultured endothelial cells and peripheral blood mononuclear cells [28, 29, 32]. These findings suggested that olive extracts, a reagent rich in antioxidants, exert its anti-atherosclerotic activity via an anti-inflammatory mechanism that may be related to anti-oxidation.

In this study, the activities of anti-oxidation and anti-inflammation on anti-atherogenesis mediated by an OLE were studied using a hyperlipidemic rabbit model. This work aimed to provide possible evidence and mechanisms of the preventive effect of OLE on atherosclerosis.

Materials and methods

Animals

Twenty-four healthy male New Zealand rabbits were from Laboratory Animal Center, Dalian Medical University. The average age of the rabbits was approximately 16 weeks (2.0–2.1 kg). At the start of treatment, rabbits were housed in single cages maintained at 24°C and 50–60% relative humidity and a 12–h light/dark cycle throughout experiment. The rabbits were acclimatized to laboratory conditions for 7 days prior to treatment.

Group composition and treatment

The animals were randomly assigned to three groups, namely control, high lipid diet (HLD), and HLD supplemented with OLE group, each group containing eight rabbits. Food intake for each rabbit was standardized to 150 g/day throughout the experiment of 6 weeks.

A special preparation of OLE extremely enriched in hydroxytyrosol, was provided by Eisai Food & Chemical Co., Ltd, Japan (Lot No.511012). The OLE powder was dissolved in tap water before use. The ingredients of OLE are hydroxytirosol (22%), polyphenol (4%), saccharide (67%), lipid (2%), ignition residue (4%) and moisture (1%).

Rabbits in the control group were given freely tap water and standard chow diet throughout the experiment, rabbits in the HLD group accepted freely tap water and high lipid diet containing 84% standard chow diet, 8% lard, 7% egg yolk powder and 1% cholesterol. Rabbits in the OLE group accepted the same feeding as that of the HLD group but tap water containing OLE to ensure the intake of OLE at 100 mg/kg of body weight.

Observations and examinations

Observations of general signs

Diarrhea, appetite and coat color were observed during the experimental period. Body weight was measured weekly.

Detection of serum lipids and malondialdehyde (MDA)

All animals were subjected to blood examination at ends of the second, fourth and sixth week. Rabbits were starved for approximately 16 h before collection of blood samples from ear vena, followed by centrifuging at 3,000 rpm for 10 min. Triglyceride (TG), total cholesterol (CHO), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were then analyzed on an automated biochemical analyzer (7060 type, HITACHI, Japan). Serum level of MDA was determined using commercial kits according to the manufacturer's instruction.

Measurement of atherosclerotic lesion area

The animals were killed at the sixth week. Rabbits were anesthetized with pentobarbital sodium (40 mg/kg) by intraperitoneal injection and received intravenous injection of heparin (400 U/kg) before they were killed by exsanguinations from the carotid artery. The aortas were exposed and cleaned of connective and adipose tissues. The abdominal aortas were removed, cut open longitudinally, stained with Sudan-IV, washed with saline, and pinned on a black paper sheet. The aortic luminal surface was photographed using a camera (DSC-S85, Sony, Japan). The surface area of lesion and whole abdominal aorta were measured by analysis of the photos. Target areas were traced by an investigator blind to the treatment and measured using Image-pro plus 5.1 (Media Cybernatics Inc). The percentage of the lesion area in aortic area was calculated as the ratio of the lesion area to the total aorta area.

Histological examination of aortic atherosclerotic lesion

In the histological examination, small fragments of the aortic arches were fixed by 10% neutral buffered formalin. The specimens were embedded in paraffin and cut serially to make cross-sectional samples. The sections were stained with hematoxylin-eosin (HE), Masson trichromic and Van Gieson's elastin. The stained sections were examined blindly to evaluate the presence of the atherosclerotic lesions. Morphological parameters were measured and quantified with Image-pro plus 5.1.

Immunohistochemistry

Paraffin-embedded aortic arches were cross-sectioned into pieces of 4 μm thick, dewaxed and rehydrated. Endogenous peroxidase activity in the sections was quenched by incubation in 3% hydrogen eroxide:methanol (1:1) for 20 min. Then, the sections were rinsed and subjected to water-bath heating for antigen retrieval. Nonspecific antibody binding was blocked by incubation of the tissue section in normal goat serum for 30 min at room temperature. Anti-NF-κB (Biochemican, USA), anti-VCAM-1 and anti- monocyte chemoattractant protein (MCP)-1 (Santa Cruz, CA, USA) were added and incubated overnight at 4°C. After being washed with PBS, a biotin-labeled secondary antibody (Zhongshan Golden Bridge Biotechnology Co., Beijing, China) was added and incubated for 30 min. The sections were then incubated with peroxidase-conjugated streptavidin for 30 min at room temperature and visualized with diaminobenzidine in darkness for 10 min. Finally, sections were counterstained with hematoxylin. In each experiment, negative controls without the primary antibody were included to check for nonspecific staining. In each section, five regions of the lesion were randomly selected to take photos. Images were quantitatively analyzed using Image Pro plus 5.1. The mean optical density was scaled by the integrated optical density (IOD) divided by area of selected region to determine an average expression level in these regions.

Reverse transcription PCR

The expression of MCP-1, VCAM-1 and TNF-α mRNA was tested using a reverse transcription PCR (RT-PCR) method. All reagents for RNA extraction and RT-PCR were from Takara Biotech (Dalian, China). Primers for MCP-1, VCAM-1, TNF-α and GAPDH were designed on the basis of the mRNA sequence in GeneBank. The primers were: 5′-GTCTCT GCAACGCTTCTGTGCC-3′ and 5′-AGTCGTGTGTTCT TGGGTTGTGG-3′ for MCP-1, resulting in a 327 bp product; 5′-GAACACTCTTACCTGTGTACAGC-3′ and 5′-CCATCCTCATAGCAATTAATGTGAG-3′ for VCAM-1, resulting in a 567 bp product; 5′-GCTCCTC ACTCACACTGTCAG-3′ and 5′-GCAATGATCCCAAAG TAGAC-3′ for TNF-α, resulting in a 246 bp product; 5′-GCGCCTGGTCACCAGGGCTGCTT-3′ and 5′-TGCC GAAGTGGTCGTGGATGACCT-3′ for GADPH, with a 465 bp product. The annealing temperature was 58°C for MCP-1, TNF-α and GAPDH, and 56°C for VCAM-1. The cycle number was 40 for MCP-1, VCAM-1, TNF-α and 32 for GAPDH.

Thoracic aorta of each rabbit were isolated, dissected, and kept in liquid nitrogen until analysis. Total RNA was extracted and reversely transcribed to cDNA according to the manufacturer's instruction. The quality of isolated RNA was examined by the ratio of A260/A280 and gel electrophoresis before use.

cDNA was synthesized by reverse transcription from 2.5 μg of total RNA. The reaction was carried out in a final volume of 25 μl. An internal control GAPDH was used to calculate the relative expressions of target genes and to standardize the cDNA loaded in each sample. The PCR products were analyzed using an automated microchip electrophoresis system as previously described. The microchip system can display electropherogram, identify peaks and calculate peak areas and heights. Same volumes of PCR product of a target gene and GAPDH was mixed and analyzed. The expression of a target gene was defined as the ratio of peak height of target gene and GAPDH [13].

Statistical analysis

All data were expressed as mean ± standard deviation. The difference of means was compared between groups. Statistical significance of difference was determined using one-way ANOVA and Student's t-test. A value of $P < 0.05$ was considered statistically significant. All statistical analysis was performed using SPSS12.0 statistical software package.

Results

General signs and changes of body weight

No remarkable changes of general appearance were observed in animals in OLE, HLD and control groups. Body weights of rabbits in OLE group did not differ from that in HLD group and control group ($P > 0.05$).

Serum lipids and MDA levels

Serum levels of CHO, HDL-C, LDL-C, TG and MDA were detected at the ends of 2nd, 4th and 6th week. In comparison with the control group, higher serum levels of CHO, HDL-C and LDL-C in HLD group were detected at the ends of 2nd, 4th and 6th week ($P < 0.01$), and elevated TG levels were detected at the ends of 4th and 6th week ($P < 0.01$). In comparison with the HLD group, rabbits in OLE group showed a reduction of CHO and LDL-C levels at the ends of 2nd, 4th and 6th week and reduction of TG level at the end of 6th week ($P < 0.05$); however, oral administration of OLE did not change the level of HDL-C in this study. In comparison with the control group higher levels of MDA were detected in HLD and OLE group at the ends of 2nd, 4th and 6th week ($P < 0.01$), and MDA levels in OLE group was significantly lower than that of the HLD group ($P < 0.05$) (Fig. 1).

Measurement of atherosclerotic lesion

Atherosclerotic lesions were found in HLD and OLE groups but not in the control group. Rabbits in the HLD group developed more serious lesions than that in the OLE group. The area of atherosclerotic lesion as a ratio to that of the whole abdominal aorta is calculated to evaluate the size of lesion. In comparison

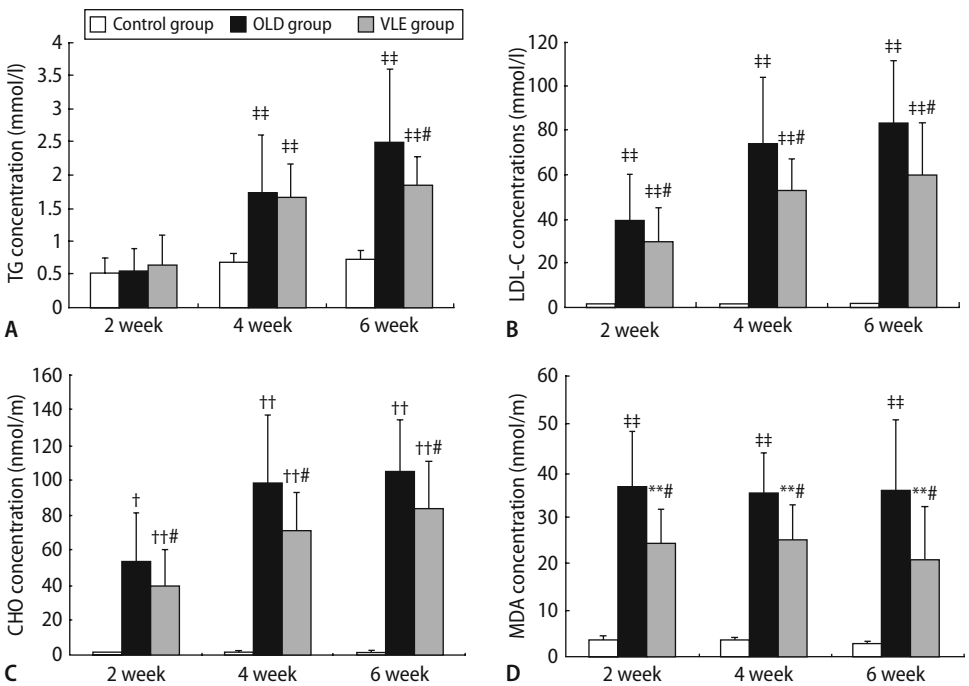

Fig. 1 Effect of OLE on serum levels of TG, LDL-C, CHO and MDA. (**a**) Effect of OLE on serum TG level; (**b**) Effect of OLE on serum LDL-C level; (**c**) Effect of OLE on serum CHO level; (**d**) Effect of OLE on serum MDA level. $\bar{x} \pm s$, $n = 8$, **Significantly different from control group at $P < 0.01$, # Significantly different from HLD group at $P < 0.05$

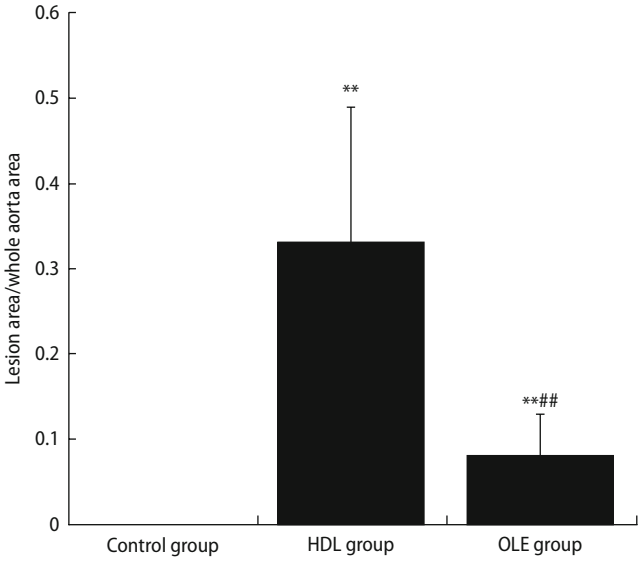

Fig. 2 Comparison of the ratio of lesion area/whole aorta area in HLD and OLE group. $\bar{x} \pm s$, $n = 8$, **Significantly different from control group at $P < 0.01$, ##Significantly different from HLD group at $P < 0.01$

with the HLD group, lesion areas in the OLE group were significantly smaller ($P < 0.05$)(Fig. 2).

Histological findings

Animals in the HLD and OLE group developed fatty streaks but no atherosclerotic lesion was observed in the control group. In the lesions, the accumulation of lipids appeared mainly in areas subjacent to endothelium. Rabbits in the HLD group developed a thick layer of lipid deposition within intima, whereas those in the OLE group showed much thinner layer of lipid deposition.

Morphological parameters of aortic arches including intimal area, medial area, ratio of intimal area/medial area and intima thickness of rabbits are shown in Table 1. The values of intimal area, ratio of intimal area/medial area and intima thickness in the HLD and OLE group were increased compared with that of the control group, and values of these parameters in the OLE group were significantly lower than those of the HLD group.

Immunohistochemistry

No or low level expression of MCP-1 and VCAM-1 was present in the control group. MCP-1 and VCAM-1 localized in endothelial cells and macrophages in the lesions. Six weeks of feeding with HLD induced severe and moderate increase of MCP-1 and VCAM-1 expression in HLD and OLE group, respectively (Fig. 3a).

Activated NF-κB was seldom detected in the control group. Significantly increased activation of NF-κB was observed in the HLD group, whereas the extent of NF-κB activation was comparably lower in the OLE group. Activated NF-κB localized mainly in endothelial cells and macrophages close to atherosclerotic lesions. In some areas, activated NF-κB was also observed in smooth muscle cells in media (Fig. 3a).

Optical density analysis showed that mean IOD in the HLD group was 0.0665 ± 0.03232 for MCP-1, 0.0134 ± 0.00637 for VCAM-1 and 0.0341 ± 0.01335 for NF-κB, while the corresponding value in the OLE group was 0.0333 ± 0.01978, 0.0067 ± 0.00512 and 0.0192 ± 0.01116, respectively (Fig. 3b). The expression of MCP-1, VCAM-1 and activated NF-κB in the HLD group was significantly higher than that of the OLE group ($P < 0.05$).

Expression of proinflammatory genes

The control group showed low level expressions of MCP-1, VCAM-1 and TNF-α, whereas these inflammation factors were increased in the HLD group. Expressions of MCP-1 and TNF-α in the OLE group were significantly down-regulated with respect to that in the HLD group. However, there was no significant difference of VCAM-1 mRNA between OLE group and HLD group (Fig. 4).

Discussion

In the present study, the anti-atherosclerotic effect of OLE and its relationship with inflammatory response were studied using an experimentally rabbit model of atherosclerosis. High lipid-diet induced hyperlipid-

Table 1 Area and thickness of intima and media in control, HLD and OLE group

	Intimal area (mm^2)	Medial area (mm^2)	Intimal area /Medial area	Thickness of intima (mm)
Control group	0	17.06 ± 5.49	0	0
HLD group	7.12 ± 6.65*	19.14 ± 5.56	0.33 ± 0.26**	0.31 ± 0.26**
OLE group	1.94 ± 0.62#	17.78 ± 4.09	0.11 ± 0.03#	0.10 ± 0.03 #

$\bar{x} \pm s$, $n = 8$, *Significantly different from the control group at $P < 0.05$; ** Significantly different from the control group at $P < 0.01$; # Significantly different from the HLD group at $P < 0.05$

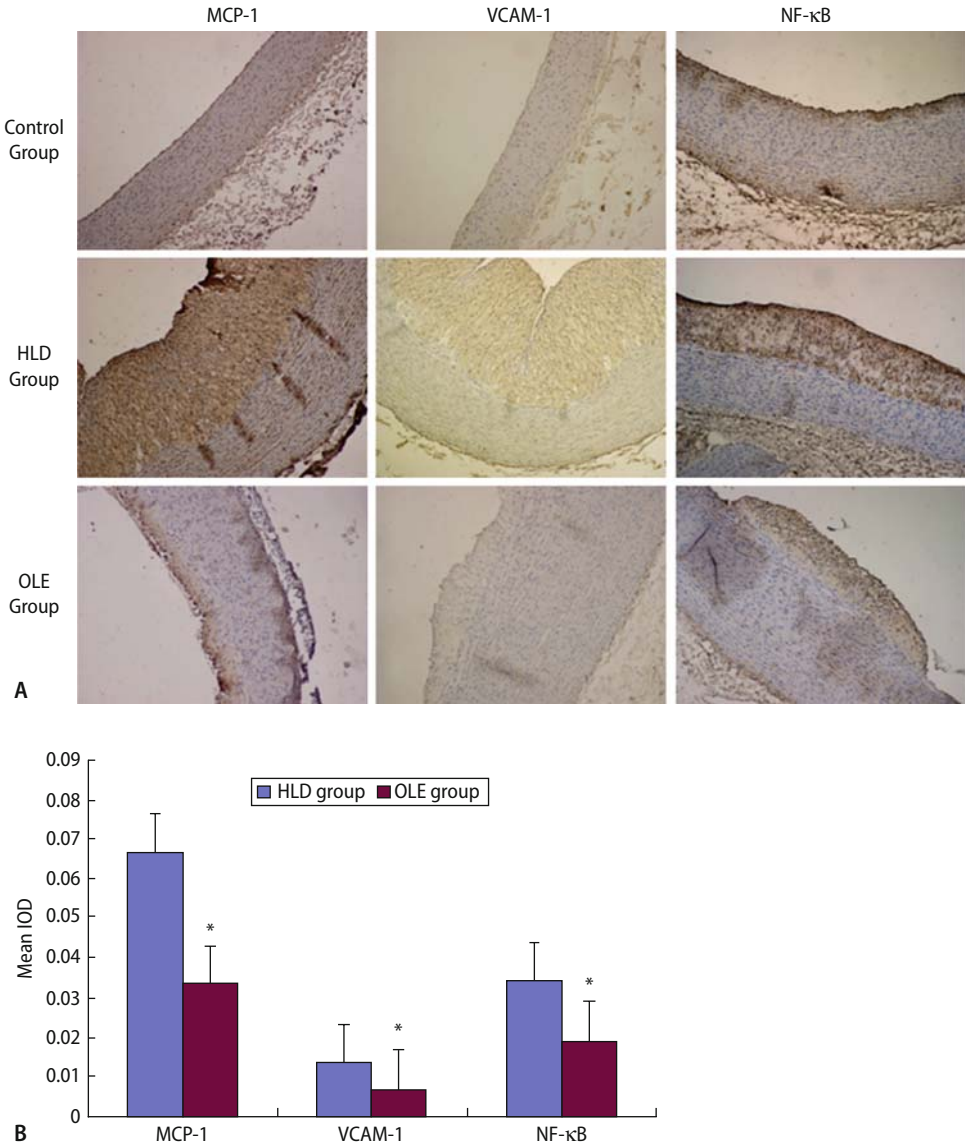

Fig. 3 Comparison of the expressions of inflammatory factors. (**a**) Representative photomicrographs of immunohistochemical examination for MCP-1 and VCAM-1 and NF-κB activation in aortas of rabbits in control group, HLD group and OLE group (×100). Little MCP-1, VCAM-1 and activated NF-κB were detected in the control group, while increased levels of MCP-1, VCAM-1 and activated NF-κB were observed in HLD and OLE group. (**b**) Comparison of mean IOD of MCP-1, VCAM-1 and NF-κB expressed in HLD and OLE group. The levels of these inflammation factors were lower in the OLE group than those in the HLD group. Data represented mean ± S.D of eight animals. *$P < 0.05$ compared to HLD group

emia and atherosclerosis in HLD and OLE group. The administration of OLE was able to decrease serum level of lipid and suppress the development of atherosclerosis. This work also revealed the role of OLE on the regulation of inflammatory factors. Hyperlipidemia induced increased levels of a series of inflammatory factors. In comparison with the HLD group, the expressions of these inflammatory factors decreased in the OLE group at both mRNA and protein level. The levels of these inflammatory factors are in accordance with the severity of atherosclerosis lesions, indicating suppression of inflammatory response could be a mechanism relevant to the anti-atherosclerotic effect of OLE.

The administration of OLE significantly reduced serum level of CHO, LDL-C and TG, but did not change HDL-C level, which was inconsistent with the previous report [20]. High levels of CHO, LDL-C and TG are risk factors for atherosclerosis whereas HDL-C has protective effects on atherogenesis, thus the selective decrease of blood lipids by OLE is favorable for inhibition of atherosclerosis.

As shown in Figs. 3 and 4, after 6 weeks of feeding with high lipid-diet, both protein and mRNA expression of MCP-1 and VCAM-1 was up-regulated in aortic endothelium, especially at atherosclerotic lesions. In contrast, supplementation with OLE resulted in significantly decreased expressions of MCP-1 at protein and mRNA level, and VCAM-1 at protein level. There was also a decrease of mRNA of VCAM-1, but there was not significant difference between OLE and HLD group. The suppression of

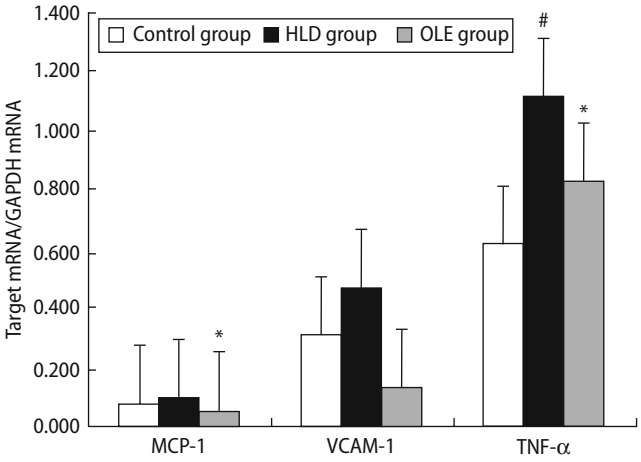

Fig. 4 Comparison of expressions of proinflammatory genes in control, HLD and OLE group. Total RNA was extracted from thoracic aorta of each rabbit and then subjected to RT-PCR using specific primers. The PCR products of proinflammatory genes, MCP-1, VCAM-1 and TNF-α, were analyzed against GAPDH using microchip electrophoresis. Expressions of the genes were shown as ratio of peak heights (target gene /GAPDH). Data represented as mean ± S.D of eight animals.* $P < 0.05$ compared to HLD group. $^{\#}P < 0.05$ compared to control group

MCP-1 and VCAM-1 is probably one mechanism by which OLE inhibits atherogenesis.

Atherosclerosis is an inflammatory disease. The thickening of the vascular wall and infiltration of macrophages and lymphocytes are hallmarks of atherosclerosis lesion [30]. At the early stage of atherosclerosis, the directive movement and transmigration of leukocytes through the endothelial layer is governed by chemotactic factors. MCP-1, a chemotactic factor with great potency for monocytes, is a glycoprotein with an apparent molecular mass of 14 kDa that synthesized and secreted by endothelial cells and smooth muscle cells upon various stimuli [21]. Our study showed the presence of MCP-1 in macrophage-rich areas (Fig. 3a), demonstrating a significant correlation between macrophage infiltration and MCP-1 expression in early lesions of atherosclerosis. Thus, decreased level of MCP-1 upon OLE administration can suppress the development of atherosclerosis by inhibiting chemotaxis. This result is in accordance with previous study using Watanabe hereditary hyperlipidemic rabbits [18].

As an initial event in the pathogenesis of atherosclerosis, local adherence of circulating monocytes to the vascular endothelial cells is one of the earliest morphological alterations. The expression of adhesion molecules, especially VCAM-1, plays a central role in the recruitment of circulating monocytes into intima. VCAM-1, one member of immunoglobulin gene superfamily, was implicated in atherogenesis by favoring firm adhesion of monocytes to vascular endothelium[12]. Some studies demonstrated that VCAM-1 plays an important role in initiation of atherosclerosis in animal studies [2, 11]. Our study showed increased levels of VCAM-1 in arterial endothelial cells mainly at lesion-prone areas and atherosclerotic plaques. The results indicated diet-induced hyperlipidemia up-regulated the expression of VCAM-1 and thus the accumulation of monocytes resulted in atherosclerotic lesions. The administration of OLE resulted in less adherence of circulating monocytes to the vascular endothelium, thus alleviate the development of atherosclerosis mediated by inflammatory response.

As a post-transcriptional regulated transcription factor, NF-κB plays an important role in increasing the expression of pro-inflammatory genes [4]. The suppressed expressions of MCP-1 and VCAM-1 prompted us to examine the influence of OLE on the activation of NF-κB. NF-κB present in cytoplasm in an inactivated form bound to its inhibitor, inhibitory κB (IκB), and is activated by detaching from IκB in response to diverse stimuli, including oxidative stress and cytokines. Activated NF-κB translocates to nucleus, where it binds to target genes and promotes transcription [8]. In this study, increased level of activated NF-κB was observed in the HLD group, especially in the areas of atherosclerotic lesions, and the administration of OLE resulted in less NF-κB activation. The results indicated that the activation of NF-κB was involved in atherogenesis and it was suppressed by OLE. OLE inhibited atherogenesis by the down-regulation of pro-inflammatory genes mediated by NF-κB.

In comparison with the control group, the level of TNF-α mRNA increased in HLD and OLE group, and the level in the OLE group was significantly lower than that in the HLD group. This result suggested that the administration of OLE inhibited the expression of TNF-α. Various biological roles have been demonstrated for TNF-α, including its involvement in inflammation and immunopathological processes. TNF-α is a modulator of the inflammatory response that occurs once the endothelium has been exposed to injurious agents. Endothelial dysfunction in atherosclerosis was elicited by TNF-α in addition to oxidative modified low density lipoprotein (Ox-LDL) [19] and increased expression of TNF-α has been found in atherosclerotic lesions [34]. The inhibition of TNF-α can decrease the binding of LDL to endothelium and smooth muscle and inhibit the transcription of LDL receptor gene [24] and the activation of T lymphocytes [22]. TNF-α was also shown to be the activator of NF-κB and have the effect of sustaining NF-κB activation [33]. As is responsible for the perpetuation of the inflammatory response, the down-regulation of TNF-α induced by OLE can inhibit the activation of NF-κB. In this study, supplementation with OLE

resulted in a significant reduction of TNF-α, which was in accordance with decreased levels of activated NF-κB, MCP-1 and VCAM-1. These findings were supported by previous reports that olive oil reduced the expression of VCAM-1 by blocking the activation of NF-κB [7]. The administration of OLE downregulated the expression of TNF-α, thus inhibited NF-κB activation which in turn decreased the expressions of MCP-1 and VCAM-1.

The oxidation status was also detected in the study by the detection of MDA, a marker indicating serum lipid peroxidation. Our results showed that the level of MDA was decreased upon OLE treatment, suggesting the effect of OLE on inhibiting the formation of lipid peroxidation. Oxidative stress is considered to be prerequisite for the endothelial dysfunction and adhesion molecules production. Injury caused by oxidation also induces inflammatory response. Previous studies showed that Ox-LDL plays crucial role in atherogenesis [38]. Olive phenols with high antioxidant capacity can thus inhibit LDL oxidation and block inflammatory response. It is reasonable to deduce that the alleviated atherosclerosis could be ascribed, at least in part, to the antioxidant capacity of OLE.

The activity of attenuating atherosclerosis with olive extracts was associated to several mechanisms including lowering lipids [20], inhibiting LDL oxidation [9], suppressing inflammatory factors [3], and preventing macrophage activation [26]. High blood lipid level causes a serial of factors contributed to inflammatory response, such as dysfunction of endothelial cell and activation of monocytes [6]. Oxidative stress induces inflammatory response by increased production of ROS and LDL oxidation [23, 37]. Therefore, the interactions of hyperlipidemia, oxidation and inflammatory response contributed to atherogenesis. The anti-atherosclerotic effect of OLE is related to the suppressed inflammatory response, which is an important mechanism in addition to the decrease of serum lipid level.

Current strategies of prevention for atherosclerosis mainly focus on lowering the level of blood lipids. Inhibition of inflammatory response and oxidation were also supposed to be potential targets for atherosclerosis prevention. Some controlled clinical trials of decreasing the level of serum lipids have not shown conclusive evidence of beneficial effects on clinical outcomes [5, 15], and pharmacologic studies showed limited effects on prevention of atherosclerosis by inhibition of inflammatory response without decreasing serum lipids [25]. The administration of OLE not only decreased the levels of blood lipid but also inhibited inflammatory response. The combined effect might account for the beneficial effects of OLE on preventing and reducing the development of atherosclerosis. This study suggests multiple-target treatment may provide a new strategy for the prevention of atherosclerosis.

References

1. Aguilera CM, Ramirez-Tortosa MC, Mesa MD, Ramirez-Tortosa CL, Gil A (2002) Sunflower, virgin-olive and fish oils differentially affect the progression of aortic lesions in rabbits with experimental atherosclerosis. Atherosclerosis 162:335–344
2. Blankenberg S, Barbaux S, Tiret L (2003) Adhesion molecules and atherosclerosis. Atherosclerosis 170:191–203
3. Bogani P, Galli C, Villa M, Visioli F (2007) Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 190:181–186
4. Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kaltschmidt C, Baeuerle PA, Neumeier D (1996) Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion J Clin Invest 97:1715–1722
5. Breslow JL (1997) Cardiovascular disease burden increases, NIH funding decreases. Nat Med 3:600–601
6. Cai H, Harrison DG (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 87:840–844
7. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De Caterina R (2003) Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol 23:622–629
8. Collins T, Cybulsky MI (2001) NF-κB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest 107:255–264
9. Covas MI, de la Torre K, Farre-Albaladejo M, Kaikkonen J, Fito M, Lopez-Sabater C, Pujadas-Bastardes MA, Joglar J, Weinbrenner T, Lamuela-Raventos RM, de la Torre R (2006) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radic Biol Med 40:608–616
10. Cullinen K (2006) Olive oil in the treatment of hypercholesterolemia. Med Health R I 89:113
11. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS (2001) A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 107:1255–1262
12. Dansky HM, Barlow CB, Lominska C, Sikes JL, Kao C, Weinsaft J, Cybulsky MI, Smith JD (2001) Adhesion of monocytes to arterial endothelium and initiation of atherosclerosis are critically dependent on vascular cell adhesion molecule-1 gene dosage. Arterioscler Thromb Vasc Biol 21:1662–1667
13. Dayu L, Xiaomian Z, Runtao Z, Nannan Y, Guohui C, Wei X, Xiaodan M, Bingcheng L (2006) Analysis of multiplex PCR fragments with PMMA microchip. Talanta 68:616–622

14. de la Puerta R, Martinez Dominguez ME, Ruiz-Gutierrez V, Flavill JA, Hoult JR (2001) Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrergic neurotransmission. Life Sci 69:1213–1222
15. de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E (2003) Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 107:690–695
16. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon diet heart study. Circulation 99:779–785
17. Dell'agli M, Maschi O, Galli GV, Fagnani R, Dal Cero E, Caruso D, Bosisio E (2007) Inhibition of platelet aggregation by olive oil phenols via cAMP-phosphodiesterase. Br J Nutr 99:945–951
18. Fruebis J, Gonzalez V, Silvestre M, Palinski W (1997) Effect of probucol treatment on gene expression of VCAM-1, MCP-1, and M-CSF in the aortic wall of LDL receptor-deficient rabbits during early atherogenesis. Arterioscler Thromb Vasc Biol 17:1289–1302
19. Gimbrone MA Jr, Cybulsky MI, Kume N, Collins T, Resnick N (1995) Vascular endothelium. An integrator of pathophysiological stimuli in atherogenesis. Ann NY Acad Sci 748:122–131; discussion 131–122
20. Gonzalez-Santiago M, Martin-Bautista E, Carrero JJ, Fonolla J, Baro L, Bartolome MV, Gil-Loyzaga P, Lopez-Huertas E (2006) One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. Atherosclerosis 188:35–42
21. Harrington JR (2000) The role of MCP-1 in atherosclerosis. Stem Cells 18:65–66
22. Hyka N, Dayer JM, Modoux C, Kohno T, Edwards CK 3rd, Roux-Lombard P, Burger D (2001) Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. Blood 97:2381–2389
23. Khan BV, Parthasarathy SS, Alexander RW, Medford RM (1995) Modified low density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells. J Clin Invest 95:1262–1270
24. Kumar A, Middleton A, Chambers TC, Mehta KD (1998) Differential roles of extracellular signal-regulated kinase-1/2 and p38(MAPK) in interleukin-1beta- and tumor necrosis factor-alpha-induced low density lipoprotein receptor expression in HepG2 cells. J Biol Chem 273:15742–15748
25. Li M, Zhang Y, Ren H, Zhang Y, Zhu X (2007) Effect of clopidogrel on the inflammatory progression of early atherosclerosis in rabbits model. Atherosclerosis 194:348–356
26. Maiuri MC, De Stefano D, Di Meglio P, Irace C, Savarese M, Sacchi R, Cinelli MP, Carnuccio R (2005) Hydroxytyrosol, a phenolic compound from virgin olive oil, prevents macrophage activation. Naunyn Schmiedebergs Arch Pharmacol 371:457–465
27. Martinez-Dominguez E, de la Puerta R, Ruiz-Gutierrez V (2001) Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. Inflamm Res 50:102–106
28. Matsubara M, Hasegawa K (2004) Effects of benidipine, a dihydropyridine-Ca^{2+} channel blocker, on expression of cytokine-induced adhesion molecules and chemoattractants in human aortic endothelial cells. Eur J Pharmacol 498:303–314
29. Perez-Martinez P, Lopez-Miranda J, Blanco-Colio L, Bellido C, Jimenez Y, Moreno JA, Delgado-Lista J, Egido J, Perez-Jimenez F (2007) The chronic intake of a Mediterranean diet enriched in virgin olive oil, decreases nuclear transcription factor kappaB activation in peripheral blood mononuclear cells from healthy men. Atherosclerosis 194:e141–e146
30. Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340:115–126
31. Rogers KA, Hoover RL, Castellott JJJ, Robinson JM, Karnovsky MJ (1986) Dietary cholesterol-induced changed in macrophage characteristics relationship to atherosclerosis. Am J Pathol 125:284–291
32. Shibata Y, Kume N, Arai H, Hayashida K, Inui-Hayashida A, Minami M, Mukai E, Toyohara M, Harauma A, Murayama T, Kita T, Hara S, Kamei K, Yokode M (2007) Mulberry leaf aqueous fractions inhibit TNF-alpha-induced nuclear factor kappaB (NF-kappaB) activation and lectin-like oxidized LDL receptor-1 (LOX-1) expression in vascular endothelial cells. Atherosclerosis 193:20–27
33. Thurberg BL, Collins T (1998) The nuclear factor-kappa B/inhibitor of kappa B autoregulatory system and atherosclerosis. Curr Opin Lipidol 9:387–396
34. Tipping PG, Hancock WW (1993) Production of tumor necrosis factor and interleukin-1 by macrophages from human atheromatous plaques. Am J Pathol 142:1721–1728
35. Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247:60–64
36. Visioli F, Galli C, Plasmati E, Viappiani S, Hernandez A, Colombo C, Sala A (2000) Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress. Circulation 102:2169–2171
37. Wang JY, Wen LL, Huang YN, Chen YT, Ku MC (2006) Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. Curr Pharm Des 12:3521–3533
38. Zheng S, Qian Z, Sheng L, Wen N (2006) Crocetin attenuates atherosclerosis in hyperlipidemic rabbits through inhibition of LDL oxidation. J Cardiovasc Pharmacol 47:70–76