Original Research Article

Synergistic anti-atherosclerotic effect of Yerba Maté (*Illex Paraguariensis*) polyphenols and Lox-1 silencing in foam cell model

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Abstract

Purpose: To elucidate the anti-atherosclerotic effect of Yerba Mate polyphenols (MP) as well as the anti-atherosclerotic effect of a combination of MP and silencing of lectin-like oxidized low-density lipoprotein receptor-1 interference group (LOX)-1.

Methods: The anti-atherosclerotic effects of control group (CG), simvastatin group (SG), MP group (MP), LOX-1 interference group (LOX) and MP + LOX-1 interference group (MP-LOX) were determined using Oil Red O staining, enzyme-linked immunosorbent assay (ELISA) and Western blot assay.

Results: The levels of foam cells, intracellular lipids, viz, total cholesterol (TC), free cholesterol (FC), cholesterol ester (CE) and acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1); LOX-1, inflammation (TNF-alpha, IL-6 and pNF-κB/NF-κB); adhesion molecular status (ICAM-1 and VCAM-1), and monocyte chemotactic protein-1 in SG and in MP, LOX and MP-LOX groups were significantly decreased, when compared with CG (p < 0.01). The levels of these parameters were much lower in MP-LOX group than in SG (p < 0.01). However, they were synergistically reduced in MP-LOX group, relative to MP group or LOX group (p < 0.01). Combination of LOX-1 gene silencing with MP produced synergistic anti-atherosclerotic effect which was reflected in decreases in foam cell formation, intracellular lipids, inflammatory status, adhesion molecular status, and MCP-1-mediated migration and infiltration of macrophages in foam cells.

Conclusion: The synergistic anti-atherosclerotic effects of MP and LOX-1 gene silencing may be potential tools for development of anti-atherosclerotic agents.

Keywords: Atherosclerosis, Adhesion, Inflammatory, Lipid accumulation, Ilex paraguariensis

INTRODUCTION

Macrophage-derived foam cells (MFC) which are chief cellular components of atherosclerotic plaques, are involved in two major pathogenic processes in atherogenesis: cholesterol buildup and inflammatory status [1,2]. Lipid droplets (LD) are the major sites of cholesterol storage in

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macrophage foam cells [3]. Increased influx and esterification of cholesterol, and decreased efflux of cholesterol result in increased accumulation of cellular cholesterol esters (CEs) [4]. When the cholesterol esters (CEs) accumulate in large amounts, they are stored as cytoplasmic lipid droplets, leading to the formation of macrophage-derived foam cells [5]. Acyl-Coenzyme A: cholesterol acyltransferase 1 (ACAT1) is an intracellular enzyme that converts free cholesterol (FC) into cholesteryl esters (CE) for storage in lipid droplets, and it promotes foam cell formation in atherosclerotic lesions [5,6].

Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are two important pro-atherosclerotic cytokines, in which TNF-α enhances macrophage foam cell formation by inhibiting intracellular lipid catabolism [7]. Intercellular cell adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-l (VCAM-1) may mediate the intimal accumulation of macrophages [8]. Monocyte chemotactic protein (MCP-1) is a crucial proinflammatory factor that contributes to the development of atherosclerotic lesions [9]. Activation of nuclear factor-kappa B (NF-κB) induces the synthesis and release of various atherosclerosis-related proteins such as inflammatory factors, adhesion molecules, and chemokines [10]. Based on the aforementioned mechanism, inflammatory status and lipid dysregulation are the key steps in the formation and development of foam cells.

Yerba Mate tea (leaves of the tree *Ilex paraguariensis*) is believed to be a natural source of cardioprotective, lipid-lowering and antioxidant compounds [11-13]. Polyphenols are the main bioactive principles of *Yerba Mate*. Previous studies have demonstrated that *Yerba Mate* polyphenols (MPs) lower blood lipid levels in people with abnormal blood lipids, and in animal models [14,15]. Simvastatin is often used to treat hypercholesterolemia, and it is effective in improving lipid profiles and reducing plasma levels of inflammatory markers. However, several studies have shown that simvastatin therapy is associated with harmful side effects [16,17]. It is still unknown whether MP inhibits foam cell formation in atherosclerosis, nor are the molecules and signaling pathways involved known.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) serves as a receptor for oxidized low-density lipoprotein (ox-LDL) [18]. The LOX-1 expressed on macrophages inhibits macrophage migration and stimulates foam cell formation, which plays a critical role in ox-LDL-mediated atherosclerosis [19]. Moreover, LOX-1

initiates a vicious cycle from activation of pro-inflammatory signaling pathways, thereby enhancing formation of reactive oxygen species and secretion of pro-inflammatory cytokines [20].

The present study was carried out to investigate the anti-atherosclerotic effect of MP and the anti-atherosclerotic effects of combination of MP and LOX-1 silencing. In the first stage, the effect of MP extract on foam cell model was studied. Then, the LOX-1 gene lentivirus interference vector was constructed, followed by determination of the influence of LOX-1 gene interference and MP on macrophage lipid metabolism and the expression levels of inflammation-related genes induced by ox-LDL.

EXPERIMENTAL

Foam cell culture

Human monocytic leukemia cell line (TH-1) was used to establish a cell model of foam cell formation induced by phorbol-12-myristate-13-acetate (PMA) and ox-LDL. The TH-1 cells were purchased from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology (320 Yue Yang Road, Shanghai 200031, PR China). The PMA (100 nmol/L) was added to human THP-1 cell culture medium for 48 h to induce the formation of macrophages. The macrophages were then incubated with ox-LDL (50 μg/Ml) for 24 h to generate foam cells.

Lentivirus-mediated LOX-1 interference

The LOX-1 gene sequence was searched in GenBank (https://www.ncbi.nlm.nih.gov/,GI: NM_001172632.1). The specific RNAi sequences targeted at human LOX-1 gene were designed using RNA interference design software (Ambion Company, Naugatuck, CT, USA). The designed siRNA was synthesized by Generay Biotech Co. Ltd (Shanghai, China). The synthesized single-stranded DNA formed double-stranded DNA oligonucleotide sequences after annealing. The linearized pLKO.1-EGFP-C1 vector was linked by Agel I and Ecol I enzyme digestion. The linearized vector was ligated with annealing shRNA, and then LOX-1 gene targeted shRNA lentivirus expression vector was constructed.

Thereafter, liposome-mediated transfection of 293T cells (packaging cells) was used to assemble pLKO.1-shLOX-1, psPAX2 and pMD2G as a complete retrovirus lentivirus particle. The 293T cells (for virus packaging) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA).

Recombinant lentivirus vector-transfected 293T cells showed that the LOX-1 protein was significantly reduced and LOX-1 lentivirus interference vector was successfully constructed.

Cell grouping and treatment

To investigate the effect of MP and LOX-1 gene silencing on ox-LDL-induced macrophage foam cell formation, ox-LDL was used to induce THP-1 cells to form foam cells in all groups. Samples of *Yerba Mate* tea were purchased from Las Marias Ltd. (Ruta Nacional, Gdor Virasoro Corrientes, Argentina). Macro-porous resin separation method was used for purification of the extract for obtaining MP [21]. Macrophage-derived foam cells were randomly divided into five sub-groups (n=6): CG which received ultra-pure water; simvastatin group (SG) treated with 100 µg/mL simvastatin; MP group treated with 200 µg/mL MP; LOX-1 interference group (LOX) treated with LOX-1 gene lentivirus interference vector, and MP + LOX-1 intervention group (MP-LOX) treated with 200 µg/mL MP and LOX-1 gene lentivirus interference vector.

After 24 h of culture, the cells were harvested and analyzed for intracellular lipid levels, including total cholesterol (TC), free cholesterol (FC0, cholesteryl ester (CE), ACAT1 and expression levels of damage response factors. Recombinant lentivirus vector-transfected 293T cells had significantly attenuated expression of LOX-1 protein, indicating that the LOX-1 lentivirus interference vector was successfully constructed.

Oil Red O staining assay

Lipid droplets in foam cells were observed with Oil Red O staining. The slides of foam cells were washed three times with phosphate buffer saline (PBS), and traces of water were blotted, followed by fixation in 10% neutral formaldehyde for 10 min, and Oil Red O staining for 10 min. The slides were decolorized with 60% isopropanol, and excessive dye was washed off. Then, the slides were washed three times with PBS, followed by counterstaining with hematoxylin for 5 min, color separation with 1 % HCl, bluing with ammonia, and mounting with gelatin glycerin. Finally, the slides were examined, photographed and recorded in a x200 field and x800 field under cx41 microscope (XDS-500C, Shanghai Caikang Optical Instrument Co. Ltd, PR China). Image J software program was used to detect and quantify foam cells stained with Oil Red O.

The percentage of foam cells was determined in terms of the relative number of cells that contained three or more fatty droplets in each field of view. A minimum of 3 fields of view were used for each experimental point. Data from six separate experiments were averaged, and each experiment had three points.

ELISA

Cells in each group were centrifuged at 1500 rpm at 4 °C for 10 min after 24 h of incubation. The concentrations of TC, FC, CE, ACAT1, TNF-α, IL-6, ICAM-1, VCAM-1 and MCP-1 in the supernatant of each group were determined using enzyme-linked immunosorbent assay (ELISA) with human ELISA kit (Wuhan Colorful Gene Biotech Company, Hubei, China), according to the manufacturer's instructions. The absorbance of each well was read at 450 nm using a trace orifice spectrophotometer. A standard curve was drawn using data produced from diluted standard solutions with curve expert 1.3. The concentrations of TC, FC, CE, ACAT1, TNF-α, IL-6, ICAM-1, VCAM-1 and MCP-1 were calculated from the standard curve. All assays were independently repeated six times.

Western blot analysis

Total protein was extracted from cells in each group using RIPA lysis buffer. Protein concentration was determined with BCA method. Then, 30-µg protein samples were resolved on SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes. The membranes were incubated in blocking buffer for 1 h at room temperature and then immunoblotted with diluted primary antibodies against LOX-1 (1:1000), glyceraldehyde-3-phosphate dehydro-genase (GAPDH) (1:1500), pNF-κB (1:800) and NF-κB (1:1000) for 12 h.

This was followed by incubation with HRP-labeled secondary antibody (1:1000) at room temperature for 1 h, and washing of the membranes using TBST. Thereafter, the bands were developed with enhanced chemilumi-nescence (ECL) and exposed in a dark room. The gray value ratio of LOX-1 and pNF-κB/NF-κB were used to indicate the relative protein expression levels of LOX-1 and NF-κB, with GAPDH as internal standard.

Statistical analysis

Statistical analysis was performed with SPSS 19.0 (IBM, Chicago, USA). All data are expressed as mean ± standard error of the mean (SEM). Comparison among multiple groups was performed using LSD analysis. Values of *p* <

0.05 were taken as indicative of significant differences.

RESULTS

Lipid droplets in foam cells

Results from Oil Red O staining showed that the PMA- and ox-LDL-induced foam cell formations were successfully established in CG. Macrophages were enlarged in size, and cytoplasm was loose and increased in size in CG. Numerous deep red-stained lipid droplets were present in cytoplasm, and the lipid content exceeded 60 % in most cells, indicating that the foam cell model was successfully established in CG. Intracellular lipid droplets, red granules and percentage of foam cells in SG, as well as MP, LOX and MP-LOX groups were significantly reduced, when compared to corresponding values in CG ($p < 0.01$). In CG group, foam cells comprised 85.95 ± 3.87 % of total cells, while the percentages of foam cells in SG, and MP, LOX and MP-LOX groups were 55.88 ± 4.1, 58.82 ± 3.92, 50.5 ± 3.21 and 33.28 ± 4.66, respectively. There were no significant differences in the percentage of foam cells between SG and MP group ($p > 0.05$). Intracellular lipid droplets, red granules and the percentage of foam cells in MP-LOX group were synergistically reduced, when compared to MP group or LOX group (Figures 1A - 1C). The results imply that LOX-1 protein expression was reduced by MP intervention or LOX-1 gene silencing, and the combination of these two resulted in synergistic effects.

Intracellular lipid assessment

The levels of intracellular lipids in SG, as well as MP, LOX and MP-LOX groups were reduced, when compared to CG. The levels of TC, FC, CE and ACAT1 in SG, and MP, LOX or MP-LOX groups were significantly decreased, relative to the CG ($p < 0.01$). The reductions in levels of TC, CE and ACAT1 in MP group were less than those in SG group ($p < 0.01$), whereas the reduction in FC was similar in SG and MP group ($p > 0.05$). The levels of TC, FC, CE and in MP-LOX group were synergistically reduced, when compared to MP group or LOX group ($p < 0.01$).

These results are shown in Table 1. The results imply that MP and LOX-1 gene silencing inhibited the expression of ACAT1, resulting in lower accumulation of CE in macrophages, reduced intracellular lipids and lower cholesterol level, while increasing cholesterol esterification and inhibiting MFC formation.
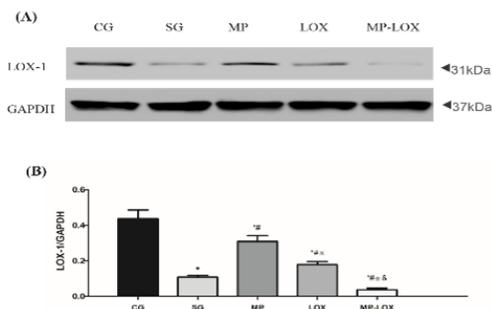
LOX-1 protein expression

To determine the protein products of LOX-1, the expressions of LOX-1 in foam cells in all groups were measured using Western blot analysis. The relative expressions of LOX-1 in SG, and in MP, LOX and MP-LOX groups were reduced, when compared to CG. There was higher level of LOX-1 in MP than in SG, which implies that the reduction in LOX-1 in MP group was less than that in SG group ($p < 0.01$).
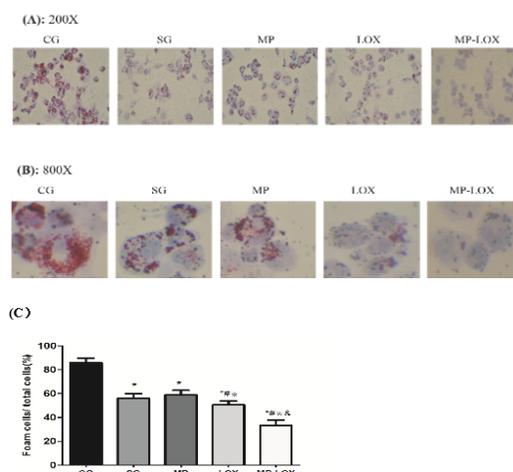
Figure 1: Lipid droplets in foam cells with Oil Red O staining. There were 5 groups in PMA/ox-LDL-induced foam cell culture: CG, SG, MP, LOX, and MP-LOX. (A) The lipid droplets in foam cells were observed in the 5 groups at x200 field under a microscope after Oil Red O staining. (B) Intracellular red granules at x800 field under microscope in the five groups. (C)The bars represent percentage of foam cells. Each experiment was performed six times with triplicate of every experimental control point. Data are presented as mean ± SE. *$p < 0.01$, compared with CG group; #$p < 0.01$, compared with SG group; ※$p < 0.01$, compared with MP group; &$p < 0.01$, compared with LOX group

Table 1: Intracellular lipids

Group	CG	SG	MP	LOX	MP-LOX
TC (nmol/mL)	49.8±1.1	36.3±0.8*	38.2±1.3* #	32.3±4.5*#※	27.4±0.8*#※&
FC (nmol/mL)	21.6±0.1	12.9±0.2*	12.2±0.2*	14.2±0.4*#※	10.4±0.2*#※&
CE (nmol/mL)	42.2±1.2	20.5±0.8*	28.0±1.3*#	26.1±4.7*#※	17.4±1.1*#※&
ACAT1 (U/L)	192±8	94±15*	156±6*#	130±15*#※	65±9*#※&

Values are mean ± SE (n = 6 for each group). There were 5 groups in PMA/ox-LDL-induced foam cell culture: CG, SG, MP, LOX, and MP-LOX. *p < 0.01, compared to CG group; # p < 0.01, compared to SG group; ※p < 0.01, compared to MP group; &p < 0.01, compared to LOX group

The relative expression level of LOX-1 in MP-LOX group was synergistically reduced, when compared to MP group or LOX group (p < 0.01) (Figure 2). These results suggest that LOX-1 gene silencing in combination with MP, produced synergistic effect on LOX-1 levels.

Figure 2: LOX-1 protein expression by western blot. The 5 groups in PMA/ox-LDL-induced foam cell culture were CG, SG, MP, LOX, and MP-LOX. (A) Representative protein products of LOX-1 extracted from foam cells in the 5 groups were measured with Western blot analysis, with GAPDH serve as internal control. (B) Bars representing the relative protein expression of LOX-1. Data presented as mean ± SE. *p < 0.01, compared with CG group; #p < 0.01, compared with SG group; ※p < 0.01, compared with MP group; &p < 0.01, compared with LOX group

Inflammatory status

To investigate inflammatory status in each group, the expressions of TNF-α and IL-6 in each group were assayed with ELISA, while pNF-κB/NF-κB was determined with Western blot analysis. The levels of TNF-α and IL-6 (Table 2) and pNF-κB/NF-κB (Figure 3) in SG, MP, LOX and MP-LOX groups were significantly decreased, when compared with their levels in CG (p < 0.01). The levels of TNF-α and IL-6 (Table 2) as well as pNF-κB/NF-κB were higher in MP than in SG (Figure 3), implying that the reductions in TNF-α and IL-6 inflammatory status in MP group were less than those in SG group (p < 0.01) (Table 2). The levels of TNF-α and IL-6 (Table 2) as well as pNF-κB/NF-κB (Figure 3) in MP-LOX group were synergistically reduced, when compared with the corresponding values in MP group or LOX group. These results suggest that LOX-1 the combination of gene silencing with MP produced synergistic anti-inflammatory effects in foam cells.

Adhesion molecular status

Compared to CG, all intervention groups had reduced levels of the adhesion molecules ICAM-1 and VCAM-1. The levels of ICAM-1 and VCAM-1 in SG, MP, LOX and MP-LOX groups were significantly decreased, when compared with CG (p < 0.01). The levels of ICAM-1 and VCAM-1 were higher in MP than in SG, which implies that the reductions in ICAM-1 and VCAM-1 status in MP group were less than those in SG (p < 0.01) (Table 2). The levels of ICAM-1 and VCAM-1 in MP-LOX group were synergistically reduced, when compared to MP group or LOX group. These results imply that combination of LOX-1 gene silencing with MP produced synergistic effects on adhesion status of foam cells.
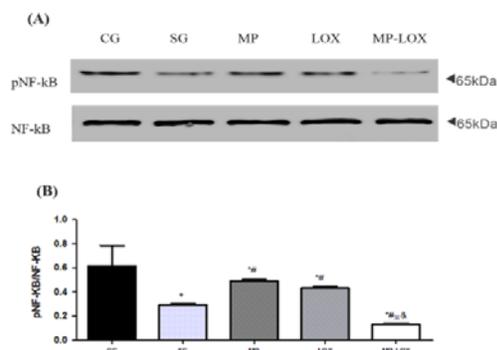
Figure 3: NF-κB protein expression levels in 5 groups of PMA/ox-LDL-induced foam cell culture: CG, SG, MP, LOX, and MP-LOX. (A) Representative protein expressions of pNF-κB and NF-κB from foam cells in the 5 groups, as measured using Western blot analysis. (B) The bars represent relative protein quantification of pNF-κB/NF-κB. Data presented as mean ± SE. *p < 0.01, compared with CG group; #p < 0.01, compared with SG group; ※p < 0.01, compared with MP group; &p < 0.01, compared with LOX group

Chemokine profiles

The levels of MCP-1 in SG, and in MP, LOX and MP-LOX groups were significantly decreased, relative to CG (p < 0.01; Table 2). The level of MCP-1 was higher in MP than in SG, which implies that the reduction in MCP-1 in MP group was less than that in SG (p < 0.01). The levels of ICAM-1 and VCAM-1 in MP-LOX group were synergistically reduced, compared to MP group or LOX group. These results indicate that combination of LOX-1 gene silencing with MP produced synergistic effects on MCP-1-mediated migration and infiltration of macrophages in foam cells.

DISCUSSION

In the present study, the effect of MP on the formation of foam cells, and the co-anti-athero-

Table 2: Intracellular damage response factors in the 5 groups of PMA/ox-LDL-induced foam cell culture: CG, SG, MP, LOX, and MP-LOX

Group	CG	SG	MP	LOX	MP-LOX
Inflammatory status					
TNF-α (pg/ml)	388±11	218±15*	320±4*#	261±4*#※	171±12*#※&
IL-6 (ng/L)	83.1±2.5	53.2±2.6*	74.9±2.9*#	61.5±3.3*#※	44.6±1.8*#※&
Adhesion molecule status					
ICAM-1 (ng/L)	449±27	268±45*	383±3*#	316±3*#※	193±4*#※&
VCAM-1 (ng/L)	36.5±0.2	23.0±0.3*	31.4±0.3*#	24.5±1.6*#※	20.4±1.4*#※&
Chemokines					
MCP-1 (ng/L)	576±3	347±21*	538±30*#	431±27*#※	283±18*#※&

Values are mean ± SE (n = 6 for each group). ELISA = enzyme-linked immunosorbent assay; TNF-α = tumor necrosis factor α; IL-6 = interleukin 6; ICAM-1 = intercellular cell adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-l; MCP-1 = monocyte chemotactic protein-1. *$p < 0.01$, compared with CG; # $p < 0.01$, compared with SG; ※$p < 0.01$, compared with MP group; &$p < 0.01$, compared with LOX group

sclerotic effect of MP and silencing LOX-1 gene, were investigated.

The results showed that MP intervention alone, and LOX-1 gene silencing alone, as well as combination of MP with LOX-1 gene silencing, all inhibited foam cell formation and LOX-1 expression. Moreover, they reduced intracellular levels of TC, FC, CE and ACAT1); decreased inflammatory status (TNF-α, IL-6 and pNF-κB/NF-κB), and reduced adhesion molecular status (ICAM-1 and VCAM-1), and MCP-1. These results indicate that the combination of MP and LOX-1 gene silencing may have pleiotropic anti-atherosclerotic synergistic effects that prevent or delay the progression of atherosclerosis. It has been reported that LOX-1 enhances vascular endothelial injury, lipid accumulation, and pro-inflammatory status in early atherosclerosis [22].

Therefore, LOX-1 gene silencing seems to be a key target that can be blocked so as to inhibit the uptake of ox-LDL by macrophages, thereby preventing the formation of foam cells. In the early stage of foam cell formation, the combination of LOX-1 with ox-LDL specifically results in unlimited uptake of ox-LDL and intracellular accumulation of a large amount of cholesterol and triglycerides. Ultimately, the cells are transformed into foam cells, followed by the formation of fatty streak [23].

This study has provided evidence showing that LOX-1 protein expression is reduced by MP or LOX-1 gene silencing. The combination of MP and LOX-1 gene silencing synergistically reduced LOX-1 protein expression. These findings suggest that MP prevents foam cell formation synergistically through decreased LOX-1, and that LOX-1 inhibition may retard atherosclerosis. Therefore, there is a certain interaction between the LOX-1 gene silencing and MP-induced beneficial synergistic effects on

LOX-1 and foam cell formation in atherosclerosis. Increased levels of intracellular lipid droplets, TC, FC, CE, ACAT1 were observed in PMA/ox-LDL-induced foam cell culture. These increases were attenuated by MP or LOX-1 gene lentivirus interference vector. The combination of MP and LOX-1 gene silencing synergistically and markedly inhibited foam cells formation and reduced intracellular lipid accumulation.

After uptake of ox-LDL by macrophages, cholesteryl esters are hydrolyzed into free cholesterol and fatty acids. The free cholesterol is esterified again under the action of ACAT1, and then stored in the cells [24]. Accumulation of a large amount of cholesteryl ester in macrophages is the key factor in atherosclerotic plaque formation [25]. In this study, MP and LOX-1 gene silencing appeared to synergistically inhibit intracellular lipid accumulation by decreasing ACAT1. The findings further support the potential link amongst MP, LOX-1, and intracellular lipids.

Inflammatory reaction is the basic pathological character of atherosclerosis and the key to its prevention. On the one hand, ox-LDL activates numerous inflammatory factors and promotes inflammatory cell infiltration. On the other hand, activated macrophage cytophagy and degraded ox-LDL lead to the accumulation of cholesterol and formation of foam cells [26,27]. These data suggest that TNF-α and IL-6 were enhanced during foam cell formation, but this effect was reversed synergistically by MP and LOX-1 gene silencing, indicating that LOX-1 inhibition and MP intervention could synergistically and markedly suppress foam cell formation.

Activated endothelial NF-κB signaling plays an important role in foam cell formation and promotes macrophage recruitment to atherosclerotic plaques. The data obtained in this

study suggest that MP and LOX-1 gene silencing could synergistically inhibit the transcriptional activity of NF-κB and its downstream factors.

Focal recruitment of monocytes is part of the earliest detectable cellular responses in atherosclerosis. The adhesion molecules ICAM-1 and VCAM-1 may enhance the movement of monocytes from blood circulation into intima, and promote the accumulation of lipids and foam on the arterial vascular wall [28]. In this study, in the PMA/ox-LDL-induced foam cells model, ICAM-1 and VCAM-1 were significantly increased. However, LOX-1 gene silencing and MP intervention synergistically and markedly reduced the levels of ICAM-1 and VCAM-1 in foam cells. Therefore, these findings imply that the combination of MP with LOX-1 gene silencing may decrease monocyte accumulation.

Studies have shown that MCP-1 attracts monocytes to the inflammatory sites of vascular subendothelial space via chemotaxis, thereby initiating the migration of monocytes into the arterial wall to form excessive amounts of macrophage-derived foam cells [29,30]. Along with macrophages, MCP-1 influences the growth of other cell types within the atherosclerotic lesion. In this study, MP and LOX-1 gene silencing synergistically and markedly inhibited MCP-1, implying that the combination of MP with LOX-1 gene silencing inhibited the migration of monocytes towards endothelium, as well as the formation of foam cells.

The limitations of this study are as follows: In the first place, this study investigated the effect of combination of MP and LOX-1 gene silencing only in a foam cell model. An animal model was not used. Therefore, there is need for the use of an animal model, as well as clinical application of combination of MP with LOX-1 downregulation in subsequent studies. Secondly, the underlying mechanism of synergistic effects of MP and LOX-1 silencing are still unclear. There is need to unravel this binding site or interaction between MP and LOX-1 because it may be a key target for treating or preventing foam cell formation.

CONCLUSION

The most important new finding in this study is that MP and LOX-1 gene silencing synergistically and markedly reduce foam cell formation, LOX-1 expression, intracellular lipids, inflammatory status, adhesion molecular status, and monocyte chemotactic protein-1. This is the first study on the synergistic effect of MP and LOX-1 gene silencing on foam cell formation. The synergistic anti-atherosclerotic effects of LOX-1 gene silencing and MP have a potential to be developed into a therapy for atherosclerosis.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shao-Hong Yu and Hui are co-first authors.

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