Department of Cardiology[1], Department of Emergency[2], Department of Neurology[3], Department of Endocrinology[4], Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai, PR China

Geniposide protects against ox-LDL-induced foam cell formation through inhibition of MAPKs and NF-kB signaling pathways

Zhi-qiang Li[1,#], Xiu-ying Huang[2,#], Chun-yan Hu[1], Zhong-sheng Zhu[1], Yang Chen[3,*], Min Gong[4,*]

Received April 15, 2019, accepted June 14, 2019

**Corresponding authors: Min Gong: Department of Endocrinology; Ynag Chen, Dpartment of Neurology, , Shanghai Pudong Hospital, Fudan University Pudong Medical Center, #2800 Gongwei Road, Huinan Town, Pudong New Area, Shanghai 201399, PR China.*
mingong163sh@163.com
[#]The authors contributed equally to this work.

Pharmazie 74: 601-605 (2019) *doi: 10.1691/ph.2019.9506*

Atherosclerosis (AS) is characterized by the significant accumulation of low-density lipoprotein (LDL)-cholesterol in macrophages that reside in the vessel wall and the resultant inflammatory response. Therefore, inhibition of LDL-induced inflammation is a promising interference for AS. Many traditional Chinese medicine prescriptions have been developed for AS treatment. Geniposide (GEN) is an iridoid glycoside mainly found in *Gardenia jasminoides* fruit. Although GEN has previously been shown to possess anti-atherosclerotic activities, its effects on the formation of macrophage-derived foam cells remain poorly characterized. In our current study, we demonstrated that GEN could significantly inhibit oxidized light-density lipoprotein (ox-LDL) induced macrophage foam cell formation and the expression of pro-inflammatory cytokines in a dose-dependent manner. In addition, treatment of GEN in bone-marrow derived macrophages repressed iNOS expression and NO expression. GEN could also alleviate ox-LDL-dependent up-regulation of CD36 expression by blocking the phosphorylation of p38 MAPK, ERK, JNK and NF-kB p65. The results of our current study demonstrate that GEN exhibits significant therapeutic effects against ox-LDA-induced foam cell formation and inflammation. Therefore, GEN is promising agent for treating AS.

1. Introduction

Atherosclerosis (AS) is an extremely prevalent pathological condition of the cardiovascular system and the leading cause of death worldwide, representing 84.5% of cardiovascular deaths and 28.2% of all-cause mortality (GBD 2013 Mortality and Causes of Death Collaborators 2015). The most prominent clinical characteristics of AS is a gradual buildup of plaques on the arterial wall (Li et al. 2017). Progressive atherosclerotic lesions can lead to thrombosis, i.e. the formation of blood clot, and the resultant partial or even complete occlusion of the affected artery (Wang et al. 2016). In severe forms of the disease, the rupture of atherosclerotic plaques plays a fundamental role in the development of potentially life-threatening myocardial infarction (Muscogiuri et al. 2017). Due to the clinical relevance of AS, the prevention and reversal of plaque formation has been a top priority of pharmaceutical research, and identification of new drug targets and discovery of new therapeutic interventions are in urgent need.

Atherosclerotic plaques consist primarily of macrophage foam cells, fat, calcium, cholesterol esters and additional cellular components. There is ample evidence that foam cells are a central contributing factor to plaque formation during the early stage of AS (Chistiakov et al. 2017). Macrophages are one of the main constituents of foam cells and contribute to formation of micro inflammatory environment in atherosclerotic plaques (Zhang et al. 2018). The injury of vascular endothelium during the early stage of AS enables the infiltration of monocytes into the subintimal space and their subsequent differentiation into macrophages (Li et al. 2016). The macrophages then ingest oxidized light-density lipoproteins (ox-LDL) and transform into cholesterol-loaded foam cells, leading to plaque formation on the side of arterial lumen (da Silva et al. 2016). In addition, pro-inflammatory mediators produced by macrophages can aggravate local inflammation and thus accelerate the progress of AS (Moore et al. 2013). Based on these findings, the macrophages in atherosclerotic lesions serve as an attractive therapeutic target for AS treatment.

Geniposide (GEN) is an iridoid glycoside mainly found in *Gardenia jasminoides* fruit and has been used as an important ingredient in many traditional Chinese medicine recipes to treat inflammation and liver diseases (Ma et al. 2015). Once ingested, GEN has been shown to be hydrolyzed into genipin by bacterial b-d-glycosidases secreted in the intestine (Akao et al. 1994). GEN has demonstrated considerable therapeutic potential against a number of pathologies, including Alzheimer's disease (Liu et al. 2015), liver diseases (Ma et al. 2015), as well as type-2 diabetes (Zhou et al. 2018). It is also found that GEN exhibits considerable protective effects against atherosclerosis by promoting the proliferation of endothelial cells and the migration of smooth muscle cells (Gao et al. 2015). However, the potential effects of GEN on the formation of macrophage-derived foam cells are still largely unknown.

In this study, we demonstrated that GEN also possesses significant atheroprotective activities by preventing foam cell formation, inhibiting macrophage uptake of ox-LDL, and alleviating AS-associated pro-inflammatory response. We are convinced that the results of our current study can help researchers further evaluate the use of GEN for AS treatment.

2. Investigations and results

2.1. Effect of geniposide on foam cell formation

Macrophage-derived foam cells play a vital role in the initiation and development of AS (Moore et al. 2013). Thus, we began the current study by investigating the effects of GEN on the transformation of macrophages into lipid-laden foam cells. As illustrated in Fig. 1A, treatment of BMDMs with up to 100 mL of GEN did not result in any significant detrimental effect on the cell viability.

We next incubated BMDMs in varying concentrations of GEN and evaluated ox-LDL-induced foam cell formation by ORO staining. As expected, the overwhelming majority of untreated BMDMs differentiated into foam cells based on the staining results (Fig. 1B). However, pretreatment with GEN was shown to suppress foam cell formation in a dose-dependent manner, as evidenced by the significantly reduced lipid uptake (Fig. 1C).

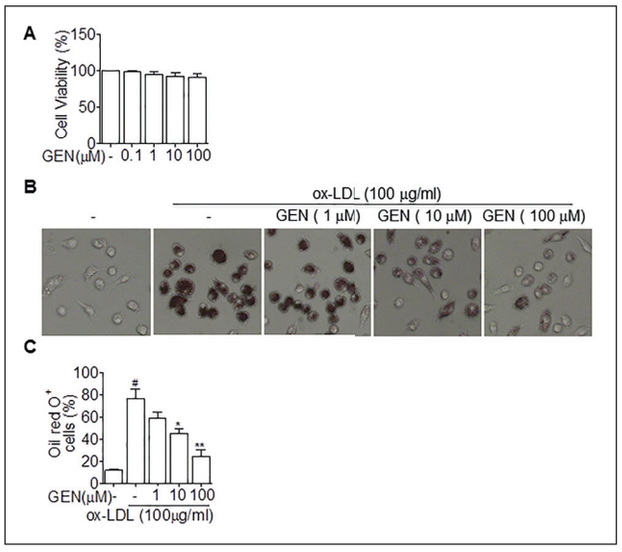

Fig. 1: Geniposide attenuates ox-LDL-induced foam cell formation. (A) Cytotoxicity in BMDMs treated with different concentrations of geniposide (GEN) (0, 0.1, 1, 10, 100 μM) for 48 h. (B) BMDMs were pretreated with GEN at 0, 1, 10, 100 μM for 1 h, and then exposed to ox-LDL (100 μg/ml) for 24 h. Form cell formation was evaluated by ORO staining and observed under a light microscope (200 ×). (C) The percentage of foam cells in the total cell population is shown. Data shown are representative images from three independent experiments (B) or are presented as the means ± SEM from three independent experiments (A, C). #P < 0.01, significantly different from the untreated group; *P < 0.05, **, P < 0.01, significantly different from ox-LDL treated group, one-way ANOVA (Bonferroni test).

2.2. Geniposide inhibited production of inflammatory cytokines

Ox-LDL has been known to promote the secretion of pro-inflammatory cytokines such as IL-6 and TNF-a, which are the hallmarks of AS-associated inflammation (Zhang et al. 2017). Based on this, we quantified the expression levels of IL-6, TNF-a, MCP-1 and KC in GEN-pretreated or untreated macrophage foam cells. As illustrated in Fig. 2A, all four cytokines were significantly upregulated in BMDMs exposed to ox-LDL in comparison to uninduced macrophages. Again, pretreatment with GEN was shown to be able to mitigate the expression of these cytokines by the lipid-laden foam cells in a dose-dependent manner (Fig. 2A). Similar trends were observed when we measured the mRNA levels of IL-6, TNF-a, MCP-1 and KC (Fig. 2B). Combined, these data demonstrated that GEN could effectively alleviate the inflammatory progression of ox-LDL-induced foam cells.

2.3. Geniposide regulates NO production and iNOS activity in foam cells

Previous studies have shown that lipids such as ox-LDL could enhance the activity of inducible nitric oxide synthase (iNOS) and resultantly lead to augmented NO production (Qin et al. 2017). Consistent with these findings, we also observed significantly increased NO level and iNOS protein expression in the ox-LDL-treated BMDMs compared to the untreated control (Fig. 3). On the other hand, GEN-pretreated BMDMs exhibited significantly lower levels of NO and iNOS in comparison to those that were not incubated with the compound prior to the lipid insult (Fig. 3).

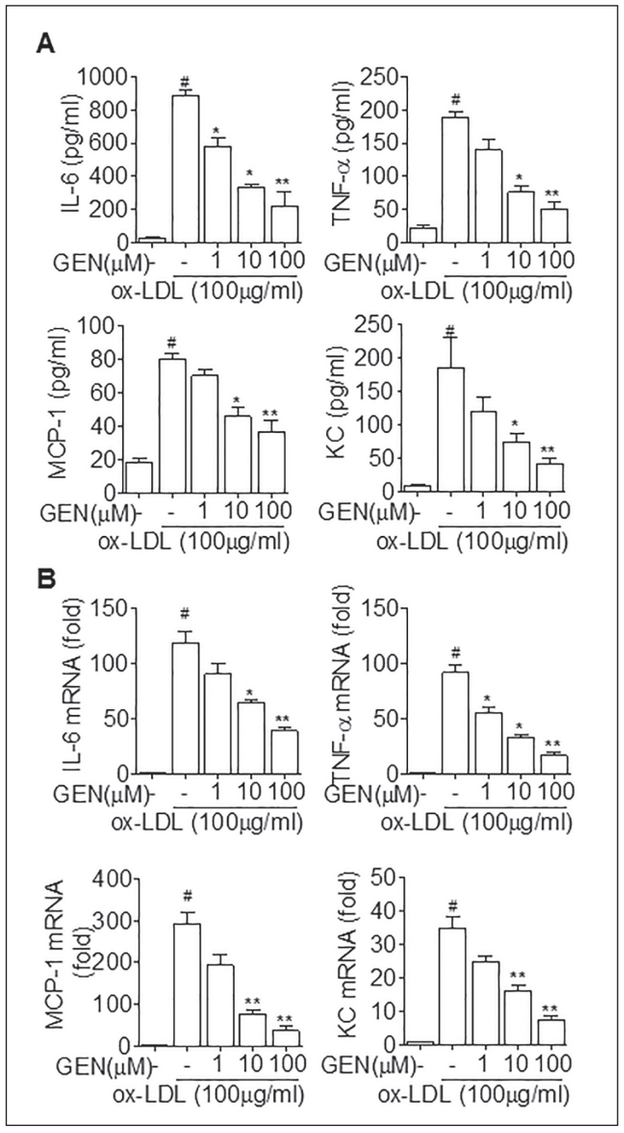

Fig. 2: Geniposide inhibits ox-LDL-induced inflammatory cytokine production. BMDMs were pretreated with GEN at 0, 1, 10, 100 μM for 1 h before stimulation with ox-LDL (100 μg/ml). After 24 hours of incubation, cells and supernatants were separately collected. The protein (A) and mRNA (B) levels of IL-6, TNF-a, MCP-1 and KC were then quantified by ELISA assay and real-time PCR respectively. The mRNA levels are shown as the relative levels of gene transcripts, with that of unstimulated cells set as 1. Data are expressed as means±SEM from three independent experiments. #P< 0.01, significantly different from the untreated group; *P < 0.05, **, P < 0.01, significantly different from ox-LDL treated group, one-way ANOVA (Bonferroni test).

Furthermore, the extent to which NO production and iNOS activity were attenuated was inversely correlated to the concentration of GEN used. Taken together, the above results suggest that GEN could alleviate the stimulation of NO production in ox-LDL-irritated macrophage foam cells.

2.4. Geniposide inhibited ox-LDL-induced CD36 expression

Macrophage CD36 is a membrane glycoprotein implicated in the pathogenic progression of AS (Park 2014). More importantly, CD36 is known to bind to ox-LDL and mediate its uptake by macrophages (Endemann et al. 1993). Conversely, internalized ox-LDL has been demonstrated to stimulate the expression of CD36 by interacting with peroxisome proliferator-activated receptor (PPAR)-γ (Nagy et al. 1998). We therefore investigated the effects of GEN on CD36 expression in BMDMs treated with ox-LDL. Consistent with the previously described results, both mRNA and protein expression

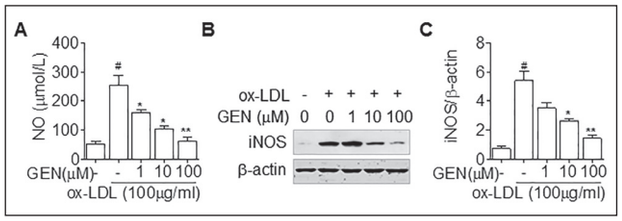

Fig. 3: Geniposide decreases ox-LDL-induced NO production and iNOS activity in foam cells. BMDMs were pretreated with GEN at 0, 1, 10, 100 μM for 1 h before stimulation with ox-LDL (100 μg/ml). After 24 h of incubation, the supernatants were collected and the NO levels were quantified (A), whereas cells were lysed for Western blot analysis. β-actin was used as loading control (B). (C) The ratio of iNOS to β-actin was analyzed by densitometry using Image-J software. All experiments were performed in triplicate and representative images are shown. #P < 0.01, significantly different from the untreated group; *P < 0.05, **, P<0.01, significantly different from ox-LDL treated group, one-way ANOVA (Bonferroni test).

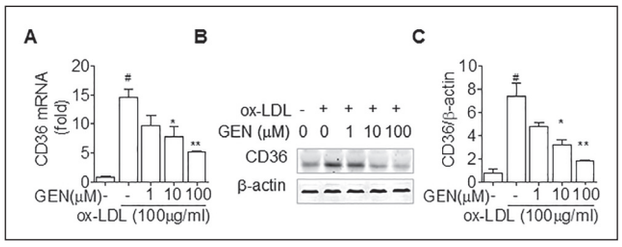

Fig. 4: Geniposide alleviates ox-LDL-induced CD36 expression. (A) BMDMs were treated with 1, 10, 100 μM GEN or 1 ‰ DMSO for 1 h before stimulation with ox-LDL (100 μg/ml) for 24 h. The CD36 mRNA levels were analyzed by real-time PCR. (B) BMDMs were treated with 1, 10, 100 μM GEN or 1‰ DMSO for 1 h before stimulation with ox-LDL (100 μg/ml) for 24 h. The protein level of CD36 was measured by Western blotting assay. (C) The ratio of CD36/ β-Actin was analyzed by densitometry using Image J software. All experiments were performed in triplicate and representative images are shown. #P < 0.01, significantly different from the untreated group; *P < 0.05, **, P < 0.01, significantly different from ox-LDL treated group, one-way ANOVA (Bonferroni test).

of CD36 was significantly up-regulated in BMDMs by ox-LDL (Fig. 4). In contrast, pretreatment with GEN significantly mitigated ox-LDL-induced CD36 expression in a dose-dependent manner (Fig. 4). Thus, the findings that we obtained provided clear evidence that GEN could downregulate CD36 in ox-LDL-loaded BMDMs, which could be associated with its role in inhibiting the formation of macrophage foam cells that we observed earlier.

2.5. Geniposide prevented ox-LDL-induced activation of mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-kB) signaling pathway

Both the MAPK and NF-kB signaling pathways play crucial roles in mediating the formation of foam cells from ox-LDL-irritated macrophages (Schwarz et al. 2017). To determine whether the MAPK and NF- kB signaling pathways were affected by ox-LDL or GEN treatment, we measured the protein levels of total and phosphorylated p38 MAPK, ERK, JNK and p65 in various BMDM experimental groups. As illustrated in Figs. 5A-D, ox-LDL exposure resulted in augmented phosphorylation of p38 MAPK, ERK and JNK in the macrophage foam cells without significantly affecting the total level of each protein. Meanwhile, GEN was found to inhibit the phosphorylation of all three proteins in ox-LDL-treated BMDMs in a dose-dependent manner (Figs. 5A-D). This suggested that GEN could effectively mitigate the ox-LDL-dependent activation of the MAPK signaling pathway. Similarly, pretreatment of BMDMs with GEN was also found to diminish the stimulatory effect of ox-LDL on the expression of p65, a key subunit of the NF-κB heterodimeric complex, in a dose-dependent manner (Figure 5E-F). Furthermore, it was also showed that GEN significantly inhibited the NF-κB luciferase activity in a dose-dependent manner in THP-1 cells (Figure 5G).

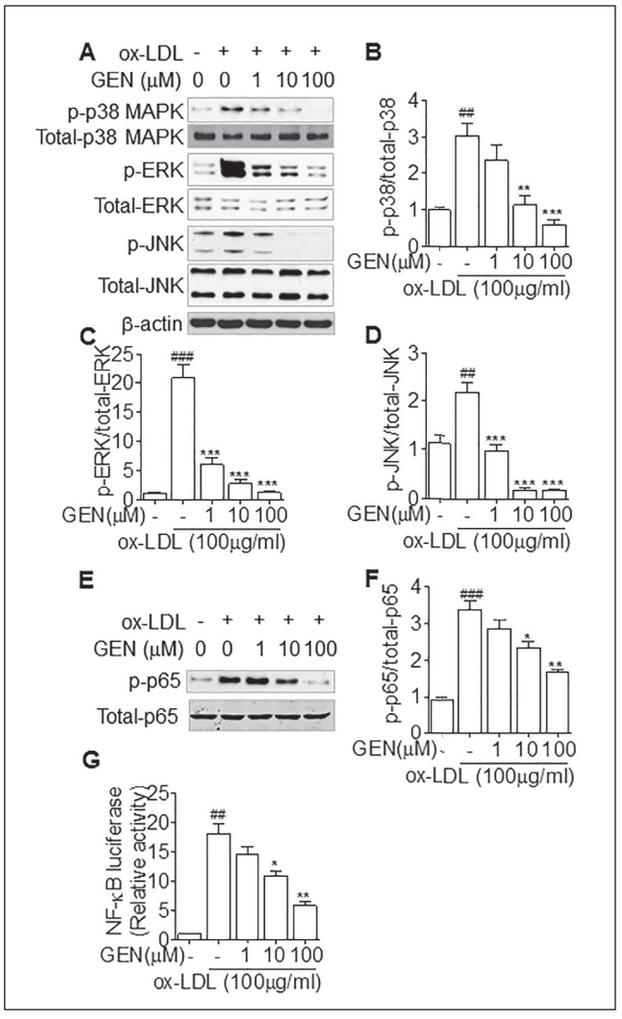

Fig. 5: Geniposide prevents ox-LDL-induced activation of MAPKs and NF-kB signaling pathway. (A) Western blot analysis of ox-LDL-induced p-p38 MAPK, p-JNK and p-ERK. BMDMs were pretreated with 1, 10, 100 μM GEN or 1 ‰ DMSO for 1 h before stimulation with ox-LDL (100 μg/ml) for 30 min. The protein levels of p-p38 MAPK, p-JNK, p-ERK and β-Actin were analyzed as above. Total p38 MAPK, JNK and ERK antibodies were used as loading controls respectively. (B-D) The ratios of p-p38 MAPK/p38 MAPK, p-ERK/ERK and p-JNK/JNK were analyzed by densitometry using Image J software. (E) Western blot analysis of ox-LDL-induced phosphorylation of p65. BMDMs were pretreated with 1, 10, 100 μM GEN or 1 ‰ DMSO for 1 h before stimulation with ox-LDL (100 μg/ml) for 30 min. The expression level of p-p65 was evaluated as above. Total p65 antibodies were used as loading control. (F) The ratio of p-p65/p65 was analyzed by densitometry using Image J software. (G) THP-1 cells were infected with NF-κB-luciferase reporter lentivirus and pretreated with GEN for 30 min. The ox-LDL-induced NF-κB activity was measured by detection of luciferase reporter. The images shown are representative of three experiments (A, E) or are presented as the means±SEM of at least three experiments (B-D and F). #P < 0.01, significantly different from the untreated group; *P < 0.05, **, P < 0.01, ***, P < 0.001, significantly different from ox-LDL treated group, one-way ANOVA (Bonferroni test).

3. Discussion

In the current study, we aimed to investigate the anti-atherosclerotic effects of GEN and to shed light on the underlying molecular mechanisms. Our results indicated that GEN suppressed ox-LDL-induced, atherogenic formation of foam cells and the release of inflammatory cytokines in a dose-dependent manner. Furthermore, it is shown that GEN could inhibit ox-LDL-mediated upregulation of CD36 and attenuate the activity of the MAPK and NF-kB signaling pathway, as evidenced by the decreased phosphorylation of p38 MAPK, ERK, JNK and p65. These data strongly suggested that GEN possesses significant anti-inflammatory and anti-atherosclerotic properties through modulation of the macrophage activation.

One of the key events in AS is the transformation of macrophages into foam cells, which are key components of plaques, as a result of their recognition and internalization of oxidized lipids (Liu et al. 2017). It is known that the endocytosis of ox-LDLs is mediated by a family of pattern recognition receptors known as scavenger receptors, which include scavenger receptor A (SRA) and CD36 (Sakai et al. 1996). CD36 is highly expressed in macrophages and its expression is regulated by multiple factors. During atherosclerosis, CD36 directly associates with ox-LDL through two of its lysine residues at positions 164 and 166 (Kar et al. 2008; Puente Navazo et al. 1996). This interaction is required for CD36 to bind to a signaling complex comprising Lyn and mitogen-activated protein kinase kinase kinase 1 (MEKK1), which then promotes the phosphorylative upregulation of JNK (Rahaman et al. 2006). Although the exact role of JNK in lipid trafficking has not been completely elucidated, studies have demonstrated that JNK inhibition could effectively block macrophage uptake of ox-LDL (Ricci et al. 2004). Additionally, p38 MAPK is also responsible for LDL-dependent downregulation of cell autophagy and the concomitant cholesterol internalization in macrophages (Mei et al. 2012), implying that there could be additional mechanisms through which the MAPK signaling pathway modulates foam cell formation. Our data show that GEN not only significantly inhibited the upregulation of CD36 but also blocked the activation of the MAPK signaling pathway. This suggested that the anti-atherosclerotic effects of GEN could at least in part be attributed to its ability to mitigate the CD36-dependent augmentation of the MAPK pathway.

In addition to its promotion of foam cell formation, ox-LDL also induces a long-lasting pro-inflammatory phenotype in macrophages, which accelerates AS by stimulating the production of pro-inflammatory cytokines. For example, ox-LDL is able to upregulate MCP-1 production by the activated endothelium (Oh et al. 2001). Mice deficient in MCP-1 and its receptor suggest that MCP-1 can induce the migration of smooth muscle cell (SMC) resulting in the retaining of SMC to the plaque regions (Kim et al. 2003). It has also been reported that enhanced secretion of TNF-a by ox-LDL-challenged macrophages plays an important role in the formation of atherosclerotic lesions (Wang et al. 2013). Our data show that GEN could inhibit the expression of TNF-a, IL-6 and MCP-1 in BMDMs that were exposed to ox-LDL. On the other hand, activation of the MAPK and NF-κB signaling pathways in foam cells is another well-established feature of AS, which, as our data indicated, could be significantly allayed by GEN. Both signaling pathways are involved in macrophage activation and inflammatory factor generation. NF-κB has been shown to modulate a variety of pathogenic features in atherosclerotic plaques. For instance, NF-κB is known to upregulate iNOS and a diverse range of pro-inflammatory cytokines such as IL-6 and TNF-α (Li et al. 2018). This is consistent with our observation that GEN-pretreated BMDMs displayed mitigated inflammation and cytokine production compared to those that were not treated by the compound prior to ox-LDL exposure. Taken together, these results suggest that the anti-inflammatory properties of GEN, particularly its suppression of the NF-κB signaling pathway, play a major role in reducing the formation of foam cells and the production of inflammatory cytokines.

Our current findings echoed those of previously published studies. For instance, administration of GEN has been shown to lower the levels of cholesterol and LPL, and attenuate the growth of atherosclerotic lesions, in mice on a high-fat diet (Wang et al. 2016). Additionally, there is evidence that GEN is capable of mitigating vascular endothelial injury by reducing epithelial cell (EC) shedding (Gao et al. 2015), which is important for decreasing lipid permeability through the affected artery walls (Kim et al. 2011). GEN-treated hyperlipidemic mice were also found to exhibit a significant drop in dickkopf-related protein-1 (DKK1) activity compared the untreated controls (Wang et al. 2016). Administration of GEN in hyperlipidemic ApoE$^{-/-}$ mice proved effective in suppressing the development of AS partly by upregulating Foxp3-positive Treg cells (Liao et al. 2014). Our current study added to the above results by demonstrating that GEN could not only suppress ox-LDL-dependent formation of foam cells from macrophages, but also diminish the production of inflammatory cytokines.

In summary, our current study offered convincing evidence that GEN could suppress ox-LDL-induced foam cell formation and inflammation in macrophages. We also demonstrated that GEN exerted an inhibitory effect on both the MAPK and NF-κB signaling pathways, which provided some mechanistic insights into its atheroprotective properties. Taken together, these results lent support to the therapeutic potential of GEN for treating AS and other cardiovascular diseases.

4. Experimental

4.1. Reagents

GEN ($C_{17}H_{24}N_{10}$; MW, 388.37) was purchased from TargetMol Co, Ltd (Shanghai, China) and HPLC analysis confirmed that its purity was > 99%. Oil Red O was obtained from Sigma-Aldrich Co. (St. Louis, MO, United States). Ox-LDL was purchased from Yiyuan Biotechnologies (Guangzhou, China). Primary antibodies against iNOS, CD36, p-p38 MAPK, p-JNK, p-ERK, p-p65, p38 MAPK, JNK, ERK, p65 and b-actin were purchased from Cell Signaling Technology (Danvers, USA).

4.2. Preparation of bone marrow derived macrophages (BMDMs)

Male C57BL/6 mice (6-10 weeks old, 20±3 g, specific pathogen free) were purchased from Shanghai Laboratory Animal Corporation (SLAC, Shanghai, China). After the mice were sacrificed, their femurs and tibias were isolated and flushed by phosphate-buffered saline (PBS) (Nie et al. 2017). Total cells were maintained for 6 days in RPMI 1640 supplemented with 2 mM of l-glutamine, 10% of fetal bovine serum (Gibco; Thermo Fisher Scientific, USA), 25 mM HEPES, 100 U/ml penicillin, 100 mg/ml streptomycin and 20 ng/ml microphage colony-stimulating factor (PeproTech, Rocky Hill, NJ). The purity of the obtained cell population was examined by fluorescence-activated cell sorting (FACS) analysis of CD11b (> 96%).

4.3. MTT assay

BMDMs were seeded in 96-well plates at a density of 4×10^4 cells per well and then incubated with different concentrations of GEN (0, 0.1, 1, 10, 100 mM) for 48 h. Next, 10 ml of 1% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (St. Louis, MO, United States) was added to each well, followed by incubation for 2-4 h. The medium was then carefully removed and 100 ml of DMSO was added to each well. The absorbance of solubilized blue formazan at 490 nm was determined by using a microplate reader.

4.4. Oil red O (ORO) staining

BMDMs were seeded into 12-well plates at a density of 1×10^6 cells per well. The cells were pretreated with varying concentrations of GEN (1, 10, 100 μM) or vehicle for 1 h, followed by treatment with 100 μg/ml ox-LDL for 24 h. Subsequently, the cells were washed, fixed with 10% formalin in PBS, and stained with 0.6% ORO (Sigma-Aldrich, USA) for 30 min at room temperature. Images of the stained cells were obtained by using a light microscope (Olympus, Japan) and analyzed with Image-J software.

4.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total mRNA was extracted from BMDMs with TRIzol reagent (Invitrogen, USA) and reverse-transcribed by using a TaKaRa Primescipt RT Reagent Kit (TaKaRa, Japan). Gene expression was quantified in triplicate on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA). Custom primers were purchased from Shanghai HuaGen Biotech. The fold change of each mRNA was calculated by the ΔΔCt method and normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

4.6. Enzyme-linked immunosorbent assay (ELISA)

The levels of interleukin-6 (IL-6), tumor necrosis factor (TNF)-a and monocyte chemoattractant protein (MCP)-1 in the cell supernatants were measured by ELISA kit (R&D Systems, USA). BMDMs were seeded in 12-well culture plates at a density of 1×10^6 cells per well and pre-incubated with varying concentrations of GEN (1, 10, 100 μM) for 1 h. Subsequently, the cells were irritated with 100 mg/mL ox-LDL for 24 h and the supernatants were analyzed by ELISA according to the manufacturer's instructions.

4.7. NO detection

Briefly, BMDMs were induced in 100 mg/mL ox-LDL for 24 h after being pretreated with or without GEN as described earlier. The supernatants were collected by centrifugation at 1000 g for 10 min and the level of NO was quantified by the Griess Reagent Kit (ThermoFisher Scientific) according to the manufacturer's protocol.

4.8. Western blotting

Whole cell protein was isolated using RIPA lysis buffer with protease and phosphatase inhibitor cocktail (Pierce, USA). The obtained proteins were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were incubated first with the indicated primary anti-

bodies overnight at 4 °C and then with the secondary antibodies for 2 h at room temperature. Finally, the stained membranes were visualized by using SuperSignal West Pico PLUS Chemiluminescent Substrate (Pierce, USA). Quantification was performed with Image J software (National Institute of Mental Health, USA).

4.9. Transfection and NF-κB-luciferase reporter assay

THP-1 cells were seeded in 12-well plates at a density of 5×10^5/well, and then cells were infected with NF-κB-luciferase reporter lentivirus (Genomeditech, Shanghai, China) for 24 h. Phorbol esters (PMA) at 200 nM were added to the medium for 24 h to transform monocytes into macrophage type cells. Then, THP-1 macrophage were treated with GEN at different concentrations for 1 h, 0.1% DMSO as solvent control, before challenged with ox-LDL (100 mg/mL) for 24 h. The cells were then analyzed using a luciferase reporter gene detection kit (Promega).

4.10. Statistical analysis

Data were expressed as mean±SEM from at least three independent experiments. Statistical significance between different samples was assessed by one-way analysis of variance (ANOVA) followed by the Bonferroni test. Statistical significance was defined as *, $P < 0.05$ and **, $P < 0.01$. Analysis and graphing were conducted using the Prism software (version 5.0; GraphPad, USA).

Acknowledgements: This study was funded by Scientific Research Projects of Science, Technology and Economy Committee of Pudong New Area, Shanghai (No. PKJ2017-Y46) and Key Specialty Construction Project of Health Commission of Pudong New Area, Shanghai (No. PWZzk2017-17).

Conflict of interest: The authors declare no competing financial interests.

References

Akao T, Kobashi K,Aburada M (1994) Enzymic studies on the animal and intestinal bacterial metabolism of geniposide. Biol Pharm Bull 17: 1573-1576.

Chistiakov DA, Melnichenko AA, Myasoedova VA, Grechko AV, Orekhov AN (2017) Mechanisms of foam cell formation in atherosclerosis. J Mol Med 95: 1153-1165.

da Silva RF, Lappalainen J, Lee-Rueckert M, Kovanen PT (2016) Conversion of human M-CSF macrophages into foam cells reduces their proinflammatory responses to classical M1-polarizing activation. Atherosclerosis 248: 170-178.

Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA (1993) CD36 is a receptor for oxidized low density lipoprotein. J Biol Chem 268: 11811-11816.

Gao Y, Chen ZY, Liang X, Xie C,Chen YF (2015) Anti-atherosclerotic effect of geniposidic acid in a rabbit model and related cellular mechanisms. Pharm Biol 53: 280-285.

GBD 2013 Mortality and Causes of Death Collaborators (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385: 117-171.

Kar NS, Ashraf MZ, Valiyaveettil M, Podrez EA (2008) Mapping and characterization of the binding site for specific oxidized phospholipids and oxidized low density lipoprotein of scavenger receptor CD36. J Biol Chem 283: 8765-8771.

Kim KI, Park KU, Chun EJ, Choi SI, Cho YS, Youn TJ, Cho GY, Chae IH, Song J, Choi DJ, Kim CH, (2011) A novel biomarker of coronary atherosclerosis: serum DKK1 concentration correlates with coronary artery calcification and atherosclerotic plaques. J Korean Med Sci 26: 1178-1184.

Kim WJ, Chereshnev I, Gazdoiu M, Fallon JT, Rollins BJ, Taubman MB (2003) MCP-1 deficiency is associated with reduced intimal hyperplasia after arterial injury. Biochem Biophys Res Commun, 310: 936-942.

Li D, Liu Y, Zhang X, Lv H, Pang W, Sun X, Gan LM, Hammock BD, Ai D, Zhu Y (2016) Inhibition of soluble epoxide hydrolase alleviated atherosclerosis by reducing monocyte infiltration in Ldlr(-/-) mice. J Mol Cell Cardiol 98: 128-137.

Li J, Xu L, Sang R, Yu Y, Ge B, Zhang X (2018) Immunomodulatory and anti-inflammatory effects of total flavonoids of Astragalus by regulating NF-KappaB and MAPK signalling pathways in RAW 264.7 macrophages. Pharmazie 73: 589-593.

Li N, Chen J, Zhao J,Wang T (2017) MicroRNA-3188 targets ETS-domain protein 4 and participates in RhoA/ROCK pathway to regulate the development of atherosclerosis. Pharmazie 72: 687-693.

Liao P, Liu L, Wang B, Li W, Fang X, Guan S (2014) Baicalin and geniposide attenuate atherosclerosis involving lipids regulation and immunoregulation in ApoE-/- mice. Eur J Pharmacol 740: 488-495.

Liu W, Li G, Holscher C, Li L (2015) Neuroprotective effects of geniposide on Alzheimer's disease pathology. Rev Neurosci 26: 371-383.

Liu Z, Zhu H, Dai X, Wang C, Ding Y, Song P, Zou MH (2017) Macrophage Liver Kinase B1 Inhibits Foam Cell Formation and Atherosclerosis. Circ Res 121: 1047-1057.

Ma TT, Li XF, Li WX, Yang Y, Huang C, Meng XM, Zhang L, Li J (2015) Geniposide alleviates inflammation by suppressing MeCP2 in mice with carbon tetrachloride-induced acute liver injury and LPS-treated THP-1 cells. Int Immunopharmacol 29: 739-747.

Mei S, Gu H, Ward A, Yang X, Guo H, He K, Liu Z, Cao W (2012) p38 mitogen-activated protein kinase (MAPK) promotes cholesterol ester accumulation in macrophages through inhibition of macroautophagy. J Biol Chem 287: 11761-11768.

Moore KJ, Sheedy FJ, Fisher EA (2013) Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 13: 709-721.

Muscogiuri G, Annweiler C, Duval G, Karras S, Tirabassi G, Salvio G, Balercia G, Kimball S, Kotsa K, Mascitelli L, Bhattoa HP, Colao A (2017). Vitamin D and cardiovascular disease: From atherosclerosis to myocardial infarction and stroke. Int J Cardiol 230: 577-584.

Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM (1998) Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell 93: 229-240.

Nie Y, Sun L, Wu Y, Yang Y, Wang J, He H, Hu Y, Chang Y, Liang Q, Zhu J, Ye RD, Christman JW, Qian F (2017). AKT2 Regulates pulmonary inflammation and fibrosis via modulating macrophage activation. J Immunol 198: 4470-4480.

Oh GT, Choi JH, Hong JJ, Kim DY, Lee SB, Kim JR, Lee CH, Hyun BH, Oh SR, Bok SH, Jeong TS (2001). Dietary hematein ameliorates fatty streak lesions in the rabbit by the possible mechanism of reducing VCAM-1 and MCP-1 expression. Atherosclerosis 159: 17-26.

Park YM (2014) CD36, a scavenger receptor implicated in atherosclerosis. Exp Mol Med 46: e99.

Puente Navazo MD, Daviet L, Ninio E, McGregor JL (1996) Identification on human CD36 of a domain (155-183) implicated in binding oxidized low-density lipoproteins (Ox-LDL). Arterioscler Thromb Vasc Biol 16: 1033-1039.

Qin M, Wang L, Li F, Yang M, Song L, Tian F, Yukht A, Shah PK, Rothenberg ME, Sharifi BG (2017) Oxidized LDL activated eosinophil polarize macrophage phenotype from M2 to M1 through activation of CD36 scavenger receptor. Atherosclerosis 263: 82-91.

Rahaman SO, Lennon DJ, Febbraio M, Podrez EA, Hazen SL, Silverstein RL (2006) A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. Cell Metab 4: 211-221.

Ricci R, Sumara G, Sumara I, Rozenberg I, Kurrer M, Akhmedov A, Hersberger M, Eriksson U, Eberli F, R, Becher B, Boren J, Chen M, Cybulsky MI, Moore KJ, Freeman MW, Wagner EF, Matter CM, Luscher TF (2004) Requirement of JNK2 for scavenger receptor A-mediated foam cell formation in atherogenesis. Science 306: 1558-1561.

Sakai M, Miyazaki A, Hakamata H, Kodama T, Suzuki H, Kobori S, Shichiri M, Horiuchi S (1996) The scavenger receptor serves as a route for internalization of lysophosphatidylcholine in oxidized low density lipoprotein-induced macrophage proliferation. J Biol Chem 271: 27346-27352.

Schwarz A, Bonaterra GA, Schwarzbach H, Kinscherf R (2017) Oxidized LDL-induced JAB1 influences NF-kappaB independent inflammatory signaling in human macrophages during foam cell formation. J Biomed Sci 24: 12.

Wang B, Liao PP, Liu LH, Fang X, Li W, Guan SM (2016) Baicalin and geniposide inhibit the development of atherosclerosis by increasing Wnt1 and inhibiting dickkopf-related protein-1 expression. J Geriatr Cardiol 13: 846-854.

Wang W, Tang Y, Wang Y, Tascau L, Balcerek J, Tong W, Levine RL, Welch C, Tall AR, Wang N (2016) LNK/SH2B3 Loss of function promotes atherosclerosis and thrombosis. Circ Res 119: e91-e103.

Wang XH, Wang F, You SJ, Cao YJ, Cao LD, Han Q, Liu CF, Hu LF (2013) Dysregulation of cystathionine gamma-lyase (CSE)/hydrogen sulfide pathway contributes to ox-LDL-induced inflammation in macrophage. Cell Signal 25: 2255-2262.

Zhang H, Zhao Z, Pang X, Yang J, Yu H, Zhang Y, Zhou H, Zhao J (2017) Genistein protects against Ox-LDL-induced inflammation through microRNA-155/SOCS1-mediated repression of NF-kB signaling pathway in HUVECs. Inflammation 40: 1450-1459.

Zhang X, Liu MH, Qiao L, Zhang XY, Liu XL, Dong M, Dai HY, Ni M, Luan XR, Guan J, Lu HX (2018) Ginsenoside Rb1 enhances atherosclerotic plaque stability by skewing macrophages to the M2 phenotype. J Cell Mol Med 22: 409-416.

Zhou Y, Men L, Pi Z, Wei M, Song F, Zhao C, Liu Z (2018) Fecal metabolomics of type 2 diabetic rats and treatment with Gardenia jasminoides Ellis based on mass spectrometry technique. J Agric Food Chem 66: 1591-1599.