Original Article

Melittin Inhibits Atherosclerosis in LPS/High-Fat Treated Mice Through Atheroprotective Actions

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Aim: Atherosclerosis is influenced by multiple environmental factors that involve a complex interaction between blood components and the arterial wall and is characterized by inflammatory reactions. Melittin has been used in treatment of various chronic inflammatory diseases. We investigated the effects of melittin regulated atherosclerotic changes in an animal model of atherosclerosis.

Methods: Atherosclerotic mice were induced by intraperitoneal (i.p) injection of lipopolysaccharide (LPS, 2 mg/kg) three times a week and an atherogenic diet for 12 weeks.

Results: Melittin (0.1 mg/kg) treatment was administered with i.p injection. Melittin treatment showed that total cholesterol and triglyceride levels decreased in atherosclerotic mice however, high-density lipoprotein cholesterol (HDL-C) levels were higher in atherosclerotic mice treated with melittin than in atherosclerotic mice. H&E staining showed that heart and descending aorta were significantly recovered by melittin, compared to atherosclerotic mice. In addition, melittin decreased the expression levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, fibronectin and transforming growth factor (TGF)- β 1 in atherosclerotic mice. *In vitro*, melittin decreased LPS-induced THP-1 cells-derived macrophages TNF- α and IL-1 β expression levels and nuclear factor (NF)- κ B signal pathway.

Conclusions: These results demonstrate that melittin has an anti-atherogenic effect by suppression of pro-inflammatory cytokines and adhesion molecules.

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Key words; Atherosclerosis, Inflammation, Melittin, Macrophage

Introduction

Atherosclerosis is a chronic inflammatory disease of the arteries resulting from interactions among lipids, monocytes and arterial wall cells. This is a complex inflammatory process that is characterized by the presence of monocytes/macrophages and T lymphocytes in atheroma¹⁾. Monocyte-derived macrophages are pivotal in atherosclerosis and are present at all stages of atherosclerosis, from nascent fatty streak lesions

to culmination in acute coronary syndromes²⁾. Moreover, macrophage density is greater in the atherosclerotic region of plaques that rupture³⁾. Macrophages are multi-potent inflammatory cells with the capacity for synthesis and secretion of pro-inflammatory cytokines. They play a central role during the development of atherosclerosis, and are one of the main cellular components of the atherosclerotic plaque^{1, 4)}; however, the mechanisms by which inflammation leads to vascular diseases have not been fully elucidated. Atherosclerosis is a slowly progressing disease, and early detection allows for more efficient treatment or even reversal⁵⁾; therefore, it is of clinical interest to be able to detect the early stages of atherosclerosis in order to select patients that will benefit from treatment.

Melittin is a major component of bee venom (BV), comprising 50% of its dry weight⁶⁾. BV therapy

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is the therapeutic application of honeybee venom to the treatment of various diseases. BV therapy has been used as a traditional medicine for treatment a variety of conditions, including arthritis, rheumatism, pain, cancerous tumors and skin diseases⁷. Many studies have examined the biological and pharmacological activities of BV. Anti-inflammatory and anti-rheumatoid arthritis effects⁸, pain-relief effect⁹, anti-cancer cell proliferation¹⁰ and immune modulatory activity¹¹ have been described for BV. BV is known to be a very complex mixture of active peptides, including melittin, phospholipase A2, apamin, adolapin and mast cell degranulating peptide¹². Melittin is a small linear peptide composed of 26 amino acid residues in which the amino-terminal region is predominantly hydrophobic, whereas the carboxyl-terminal region is hydrophilic. The structure of melittin is tetrameric at the concentrations prevailing in the venom sac of the bee and monomeric at the minimum concentrations needed for cell lysis¹³. These characteristics may play an important role in the anti-inflammatory effects of melittin.

Several experimental studies have shown that melittin therapy attenuates atherosclerosis^{14, 15}; however, systematic experiments demonstrating the molecular mechanisms of the anti-atherosclerotic effects of melittin in mouse models of atherosclerosis have not been reported. In this study, we investigated the protective effects of melittin on serum lipid profiles, pro-inflammatory cytokines, pro-atherogenic proteins and adhesion molecule levels in a mouse model of atherosclerosis and monocyte-derived macrophages.

Materials and Methods

Melittin Collection

Melittin was isolated from honeybee (*Apis mellifera*) venom. Purified melittin was obtained from two experimental colonies maintained at the National Institute of Agricultural Science and Technology, Suwon, Korea¹⁶. Dried melittin was dissolved in distilled water and centrifuged at 8,000 g for 10 minutes to remove insoluble materials. Melittin was lyophilized by freeze drying and refrigerated at 4°C for later use.

Experimental Model

Male C57/BL6 mice (Orient, Busan, Korea) were used in this study. Mice were maintained in a room set at 21–15°C with a 12 h light/dark cycle. They were given an intraperitoneal injection of LPS (2 mg/ml; Sigma St Louis, Mo) three times a week to in-

duce acute atherosclerosis, and fed an atherogenic diet (1.25% cholesterol, 15% fat and 0.5% cholic acid) for 12 weeks starting at 8 weeks of age. The mice were fed diet (normal control, NC, $n=15$), atherogenic diet (LPS/Fat, $n=15$) and atherogenic diet melittin treatment (LPS/Fat + Mel, $n=15$). Melittin (0.1 mg/kg) was given an intraperitoneal injection twice a week after LPS treatment. Five mice in each group were sacrificed 4, 8 and 12 weeks after LPS treatment in mice fed the atherogenic diet.

Serum Lipid Analysis

Blood samples were collected at the time of sacrifice. Serum concentrations of TC, TG and HDL-C were determined enzymatically using commercially available kits (Asan, Hwaseong, Korea).

Pro-Inflammatory Cytokines

The serum levels of TNF- α and IL-1 β assay were assessed using the ELISA Kit (R&D Systems, Minneapolis, MN). The absorbance at 540 nm was determined using a microplate reader.

Protein Isolation and Western Blot Analysis

The heart and aorta tissues and cells were homogenized in lysis buffer (50 mM pH 8.0 Tris, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 100 mM PMSE, 1 mg/ml leupeptin, 1 mg/ml aprotinin, and 1M DTT). Protein contents were measured using a Bio-Rad colorimetric protein assay kit (BioRad, Hercules, USA). Protein was separated on 6–10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred onto a polyvinylidene fluoride membrane (PVDF, Millipore, MA, USA). The membranes were blocked with a 5% skim milk solution for 1 h. Protein samples were incubated overnight with anti-VCAM-1 (1:1000; R&D Systems, MN, USA), anti-ICAM-1 (1:1000; R&D Systems), anti-TGF- β 1 (1:500; R&D Systems), anti-fibronectin (1:1000), phospho-IKK (1:1000; Cell Signaling Technology, MA, USA), anti-phospho-I κ B (1:1000; Cell Signaling Technology), anti-phospho-NF- κ B p65 (1:1000; Cell Signaling Technology) and anti-glyceraldehyde-3-phosphate-dehydrogenase (GAPDH, 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as the primary antibodies, respectively. The membranes were washed several times with TBS-T and incubated in a horseradish peroxidase (HRPO)-conjugated secondary antibody (1:2000; Amersham Biosciences Abcam, MA, USA, NJ, UK (Author: This is a combination of two companies/countries - which is correct?)). Protein expression levels were detected by enhancing chemiluminescence (ECL) with X-ray film and ECL reagents

Fig. 1. Histological observation of the aorta (A) and heart (B) from atherosclerotic mice treated with melittin

Advanced fatty plaques containing lipid-rich components were present in the aorta and heart from the LPS/Fat group. Melittin treatment notably reduced the progression of atherosclerotic lesions. NC, normal control; LPS/Fat, LPS injection and high-fat diet mice (atherosclerotic mice); LPS/Fat + Mel, atherosclerotic mice treated with melittin. * $P < 0.05$ versus NC group; # $P < 0.05$ versus LPS/Fat group. Scale bars, 50 μm .

(Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Signal intensity was quantified by an image analyzer (Las 3000; Fuji, Japan).

Atherosclerosis Analysis (Histological, Immunohistological and Immunofluorescence Analysis)

The aorta and heart specimens were fixed overnight in 10% formalin solution then dehydrated, embedded in paraffin, and cut into 4 μm sections. Sections were stained with hematoxylin and eosin (H&E). For immunohistochemical analysis, sections were processed by an indirect immunoperoxidase technique using a commercial kit (LSAB kit; DakoCytomation, Carpinteria, CA, USA). The sections were incubated with anti-VCAM-1, anti-ICAM-1, anti-TGF- β 1, anti-fibronectin and anti-F4/80 antibodies for 1 h. Immunofluorescence staining of the paraffin sections for anti-F4/80 (1:200; Abcam) was performed to confirm the presence of macrophages in vascular walls and plaques. The protocol was performed as described previously. The sections were incubated with fluorescently labeled secondary antibody (FITC-conjugated AffiniPure goat anti-mouse IgG; Jackson ImmunoResearch Laboratories Inc., PA, USA) for 30 min. The sections were counterstained with propidium iodide (PI). Light microscopy was used to acquire immunofluorescent images.

Cell Culture

The human monocytic cell line THP-1 was obtained from the American Type Culture Collection and cultured with RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics. Cells were cultured in a humidified incubator at 37°C

in a 5% CO₂ atmosphere. THP-1 cells (1×10^6 cells/ml) were differentiated to macrophages using 50 nM phorbol 12-myristate 13-acetate for 48 h. THP-1 cell-derived macrophages incubated in serum-free culture medium prior to treatment with melittin (0.1, 0.5, 1 $\mu\text{g/ml}$) for 6 h. After incubation with melittin, THP-1 cell-derived macrophages were activated with LPS (1 $\mu\text{g/ml}$) for 24 h. Supernatants were collected and stored at -80°C until quantification of TNF- α and IL-1 β expression. These were measured by ELISA (R&D Systems, Oxon, UK) according to the manufacturer's instructions.

Statistical Analysis

The data were analyzed using one-way ANOVA and expressed as the mean $\pm$ S.D. Significant differences between groups were detected by SPSS 12.0 software. $p < 0.05$ was considered significant. All experiments were performed at least three times.

Results

Histopathological Analysis

Pathological evaluations of atherosclerotic lesions, lipid depositions and fatty streaks were made in atherosclerotic mice. Histopathological changes of aortic arch dissection were prominent in the descending aorta and heart, as seen after staining with H&E (Fig. 1). After treatment with LPS injection and a high-fat diet for 4, 8 and 12 weeks, atherosclerotic changes were observed in the aorta and heart of all experimental groups. The LPS/Fat group showed thickened arterial walls and larger atherosclerotic lesions in the heart than the NC group. Treatment with melittin

Fig. 2. Serum lipid levels of experimental mice

TC (A) and TG (B) levels increased in the LPS/Fat group. Melittin significantly decreased TC levels and TG levels in the LPS/Fat group. HDL-C (C) levels increased in LPS injection and high-fat diet mice by melittin. NC, normal control; LPS/Fat, LPS injection and high-fat diet mice (atherosclerotic mice); LPS/Fat + Mel, atherosclerotic mice treated with melittin. Data are the means $\pm$ S.D. ($n=5$). * $P < 0.05$ versus NC group; # $P < 0.05$ versus LPS/Fat group.

Fig. 3. Serum levels of pro-inflammatory cytokines in atherosclerotic mice

TNF- α (A) and IL-1 β (B) levels in serum were determined by ELISA. After treatment with melittin, expression levels of TNF- α and IL-1 β were reduced compared with the LPS/Fat group. NC, normal control; LPS/Fat, LPS injection and high-fat diet mice (atherosclerotic mice); LPS/Fat + Mel, atherosclerotic mice treated with melittin. Data are the means $\pm$ S.D. ($n=5$). * $P < 0.05$ versus NC group; # $P < 0.05$ versus LPS/Fat group.

resulted in markedly decreased development of atherosclerotic lesions in the aorta and heart compared with the LPS/Fat group (**Fig. 1**); therefore, histological examination confirmed that melittin exerts apparent protective atherogenic actions.

Serum Lipids

To examine the effect of melittin on serum lipid levels, we measured serum levels of TC, TG and HDL-C before and during 4, 8 and 12 weeks after LPS injection and a high-fat diet. Initial TC concentration in serum exhibited approximately the same values in the LPS/Fat and LPS/Fat + Mel groups; however, the serum TC was gradually decreased by melittin (**Fig. 2A**). The concentration of serum TG also showed a significant reduction after 8 and 12 weeks of LPS and high-fat feeding by melittin, respectively, as

compared to the LPS/Fat group (**Fig. 2B**). The serum HDL-C concentration was significantly higher in the LPS/Fat+Mel group than in the LPS/Fat group (**Fig. 2C**). Thus, melittin controls serum lipid levels in atherosclerotic mice.

Pro-Inflammatory Cytokines

TNF- α and IL-1 β were determined in serum by ELISA. The initial expression levels of the two inflammatory factors were higher in the LPS/Fat group than in normal control mice. After 4, 8 and 12 weeks of melittin treatment, the LPS/Fat + Mel group had lower expression levels of serum TNF- α and IL-1 β than the LPS/Fat group (**Fig. 3**). These results indicate that melittin inhibits TNF- α and IL-1 β expression at the serum level in atherosclerotic mice.

Fig. 4. Expression levels of adhesion molecules and fibrotic pro-atherogenic proteins by melittin in the aorta of atherosclerotic mice

(A) Expression levels of VCAM-1, ICAM-1, fibronectin and TGF- β 1 in Western blotting were decreased by melittin. GAPDH levels were used as internal markers for loading variation. Lower panels show the band density of the upper panels. (B) Representative section of immunohistochemical staining for expression levels of VCAM-1, ICAM-1, fibronectin, TGF- β 1 and macrophages in the aorta of atherosclerotic mice at 12 weeks. Treatment with melittin resulted in a significant decrease of these proteins in the aorta of atherosclerotic mice. NC, normal control; LPS/Fat, LPS injection and high-fat diet mice (atherosclerotic mice); LPS/Fat + Mel, atherosclerotic mice treated with melittin. Data are the means $\pm$ S.D. ($n=5$). * $P<0.05$ versus NC group; # $P<0.05$ versus LPS/Fat group. Scale bars, 50 μ m.

Adhesion Molecules and Proatherogenic Proteins

To examine the effect of melittin on protein expression levels, we determined protein levels of adhesion molecules (VCAM-1 and ICAM-1) and proatherogenic proteins (fibronectin and TGF- β 1) before and during 4, 8 and 12 weeks after LPS injection and a high-fat diet. As shown in **Fig. 4A** and **5A**, the aorta and heart from the NC group showed little expression of VCAM-1, ICAM-1, fibronectin and TGF- β 1 protein; however, more significant up-regulation of VCAM-1, ICAM-1, fibronectin and TGF- β 1 expression was observed in the aorta and heart from the LPS/Fat group, while treatment with melittin led to evident down-regulation of these protein expression levels. The effect of melittin on atherosclerosis development was also assessed in cross-sectional lesions of the aorta and heart. The content of interstitial VCAM-1, ICAM-1, fibronectin and TGF- β 1 was re-

duced by melittin treatment at 12 weeks, as determined by immunohistochemistry. These findings were consistent with Western blot examination of VCAM-1, ICAM-1, fibronectin and TGF- β 1 expression (**Fig. 5B** and **5B**). In addition, macrophage infiltration was attenuated by melittin, as determined by anti-F4/80 immunohistochemistry (**Fig. 5B**). **Fig. 6** shows macrophage infiltration detected by immunofluorescence staining. We did not observe any change in mice fed a chow diet. In all other experimental groups, atherosclerotic lesions exhibited a significant decrease in macrophage infiltration in the aorta and heart from the LPS/Fat + Mel group compared to the LPS/Fat group.

THP-1 Cell-Derived Macrophage Cytokine Expression

In order to investigate the question of whether

Fig. 5. Expression levels of adhesion molecules and fibrotic pro-atherogenic proteins by melittin in the heart of atherosclerotic mice

(A) Expression levels of VCAM-1, ICAM-1, fibronectin and TGF-β1 in Western blotting were decreased by melittin. GAPDH levels were used as internal markers for loading variation. Lower panels show the band density of the upper panels. (B) Representative section of immunohistochemical stain for expression levels of VCAM-1, ICAM-1, fibronectin, TGF-β1 and macrophages in the heart of atherosclerotic mice at 12 weeks. Treatment with melittin resulted in a significant decrease of these proteins in the heart of atherosclerotic mice. NC, normal control; LPS/Fat, LPS injection and high-fat diet mice (atherosclerotic mice); LPS/Fat + Mel, atherosclerotic mice treated with melittin. Data are the means $\pm$ S.D. ($n=5$). * $P<0.05$ versus NC group; # $P<0.05$ versus LPS/Fat group. Scale bars, 50 μ m

Fig. 6. Identification of macrophages in atherosclerotic lesions

Representative sections of aorta (A) and heart (B) stained with antibodies specific to macrophages in atherosclerotic mice at 12 weeks. Macrophage infiltration was reduced by melittin. Micrographs display macrophages (green), nuclei (red) and merged image. NC, normal control; LPS/Fat, LPS injection and high-fat diet mice (atherosclerotic mice); LPS/Fat + Mel, atherosclerotic mice treated with melittin. Scale bars, 50 μ m

Fig. 7. LPS-induced expression levels of pro-inflammatory cytokines in THP-1 cell-derived macrophages

Expression levels of TNF- α (A) and IL-1 β (B) in the culture supernatant were determined by ELISA. Expression levels of TNF- α and IL-1 β were decreased by melittin. Data are the means $\pm$ S.D. of three experiments. * $P < 0.05$ versus normal control; # $P < 0.05$ versus LPS alone.

melittin has an effect on the inflammatory response, THP-1 cell-derived macrophages were selected as a model system. We preliminarily compared melittin isolated from bee venom and recombinant melittin with TNF- α level by Western blotting. We showed that anti-inflammatory effects of melittin isolated from bee venom are higher than with recombinant melittin (data not shown). For our preliminary results, we used melittin, which is isolated bee venom, in this experiment. We assessed the effect of melittin on LPS-induced pro-inflammatory cytokine expression in THP-1 derived macrophages (**Fig. 7**). Expression of TNF- α and IL-1 β was significantly increased by treatment with LPS at a concentration of 1 μ g/ml; however, when these cells were pretreated with melittin (0.1, 0.5 and 1 μ g/ml), the expression of each of these cytokines in response to LPS was reduced. We found a significant inhibitory effect of melittin on the expression of TNF- α and IL-1 β in LPS-treated THP-1 cell-derived macrophages.

THP-1 Cell-Derived Macrophage NF- κ B Activation

NF- κ B is an important transcription factor for stimulation of various inflammatory genes in macrophages treated with LPS¹⁷). To determine whether IKK, I κ B and NF- κ B signaling pathways were involved in the anti-inflammatory effects of melittin, activation of these three proteins was examined by Western blotting of their phosphorylated forms. As shown in **Fig. 8**, melittin suppressed the phosphorylation of IKK and I κ B in LPS-treated THP-1 cell-derived macrophages. In addition, inhibition of IKK and I κ B phosphorylation resulted in decreased nuclear translocation of active p65 in LPS-treated THP-1 cell-derived macrophages indicating suppression of NF- κ B transcriptional activation. These results demonstrated

that melittin regulates inflammatory reactions through the NF- κ B signal pathway in LPS-treated THP-1-derived macrophages.

Discussion

In this study, we investigated the effect of melittin on the progression and instability of atherosclerotic lesions in mice fed a high-fat diet with LPS induction. The major finding of the present study is that melittin prevents LPS/high-fat-induced expression levels of inflammatory cytokines, proatherogenic proteins and adhesion molecules. This suggests that melittin may play a significant role in atherosclerosis.

Increased serum cholesterol and triglyceride is known to be a major risk factor related to the development of atherosclerosis¹⁸). Cholesterol and triglycerides all contain fatty acids. Lipoprotein lipase (LPL) anchored to endothelial cells, resulting in the release of free fatty acids¹⁹). Pilz *et al.*²⁰) have reported that free fatty acids (FFA) are independently associated with cardiovascular disease, indicating that FFA might promote the development of atherosclerosis. Although FFA is linked to the pathogenesis of atherosclerosis²¹), how FFA causes atherosclerosis has not been determined²²). Further study is needed to understand the mechanism of FFA regulation of melittin both *in vitro* and *in vivo*.

The severity of atherosclerosis is positively correlated with the concentration of circulating TG-rich lipoproteins, and negatively related to HDL-C levels²³). Reduction of serum TC plays a major role in mediating the repression of atherosclerosis²⁴). Of particular interest, we showed that the major changes resulting from treatment with melittin appeared to be a decrease in TC and TG concentrations, and an increase

Fig. 8. Expression levels of the NF- κ B signaling pathway in LPS-treated THP-1 cell-derived macrophages

Expression levels of IKK and I κ B in the cytosolic fraction and NF- κ B (A) in the nuclear fraction were determined by Western blot, densitometry of Western blot (B, C and D). GAPDH levels were used as internal markers for loading variation. Expression levels of IKK, I κ B and NF- κ B phosphorylation were decreased by melittin. Data are the means $\pm$ S.D. of three experiments. * P < 0.05 versus normal control; # P < 0.05 versus LPS alone.

in the HDL-C concentration in the serum of atherosclerotic mice. Elevation of serum lipids, which results from lipid metabolism disorder, plays an important role in the initiation of atherosclerosis, and lipid metabolism was affected by cytokines. Serum cholesterol has been reported to elevate the expression of TNF- α and IL-1 β by increasing cholesterol synthesis²⁵.

Of particular importance with regard to atherosclerosis is the induction of pro-inflammatory cytokines, which recruit inflammatory cells to loci of inflammation²⁶. TNF- α and IL-1 β , a key pro-inflammatory cytokine, as part of the inflammatory cascade, are considered to play important roles in atherogenesis, and are produced by several types of inflammatory cells, including macrophages, vascular endothelial cells and smooth muscle cells. Recruitment of inflammatory cells plays a primary role during the early stage of atherosclerosis²⁷. Recently, numerous basic research studies have indicated that TNF- α accelerates atherosclerosis in mice^{28, 29}. Moreover, IL-1 β , which plays an important role in the mediation of inflammatory

responses and in the pathogenesis of atherosclerosis, is secreted by macrophages in atherosclerotic lesions³⁰. Thus, we investigated the question of whether melittin could affect two pro-inflammatory cytokines. We found that LPS injection and a high-fat diet increased serum levels of TNF- α and IL-1 β in mice; however, melittin supplementation decreased serum levels of TNF- α and IL-1 β in atherosclerotic mice.

Pro-inflammatory cytokines could up-regulate the expression of adhesion molecules, chemokines and scavenger receptors in the aorta³¹. Adhesion molecules such as VCAM-1 and ICAM-1 play a significant role in the process of atherosclerosis as they ensure the recruitment of inflammatory cells. While VCAM-1 is primarily an inducible molecule, ICAM-1 is also constitutively expressed on resting endothelial cells; both molecules are up-regulated by pro-atherogenic factors. High levels of both VCAM-1 and ICAM-1 in serum have been linked to risk of cardiovascular events³². The lesion of atherosclerotic change was accompanied by upregulation of the expression levels of VCAM-

1 and ICAM-1 measured by immunohistochemistry. In general, a high-fat diet further induced both lesions of atherosclerotic change and expression levels of VCAM-1 and ICAM-1³³; this corresponds well with our results. In addition, we found that melittin significantly decreased expression levels of VCAM-1 and ICAM-1 in atherosclerotic mice.

Endothelial cells, macrophages and smooth muscle cells all contribute to extracellular matrix (ECM) production. As atherosclerosis progresses, the presence of many atherogenic cytokines stimulates SMC to favor the production of fibronectin as well as to enhance the rate of ECM synthesis³⁴. ECM makes up fatty streaks and atherosclerotic plaques³⁵. Consistently, we showed that LPS injection and a high-fat diet increased fibronectin production in atherosclerotic mice, and that melittin markedly suppressed the expression of fibronectin.

Activation of mononuclear cells in the arterial walls is a critical step in atherogenesis. TNF- α and IL-1 β contribute to the activation of many signal transductions, leading to atherosclerosis^{29, 36}. We found that LPS (1 μ g/ml) stimulation increased TNF- α and IL-1 β from THP-1-derived macrophages, which was significantly reduced by treatment with melittin.

Activated IKK phosphorylates the endogenous NF- κ B inhibitor, I κ B, leading to its degradation and subsequent nuclear translocation of NF- κ B proteins, including p65³⁷. Our results showed that IKK and I κ B phosphorylation in LPS-treated-THP-1 cell-derived macrophages was decreased by melittin. Moreover, nuclear NF- κ B phosphorylation was suppressed by melittin in a dose-dependent manner; therefore, we showed that melittin exerts its antiatherosclerotic effects partly via the inhibition of lipid levels, pro-inflammatory cytokines, adhesion molecules, pro-atherogenic proteins and the NF- κ B signal pathway.

Conclusion

In summary, our results confirmed that melittin decreased atherosclerotic lesions dependently on serum lipid levels. The mechanisms are partly attributable to the inhibition of atherosclerotic changes with downregulation of pro-inflammatory cytokines, adhesion molecules, pro-atherogenic proteins and the NF- κ B signal pathway. Our findings show part of the molecular mechanism of melittin in the treatment of atherosclerosis. Melittin has a significant role in atherogenesis, and its inhibition may be therapeutically useful in the prevention of atherosclerosis.

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