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Atorvastatin Suppresses Inflammatory Response Induced by oxLDL Through Inhibition of ERK Phosphorylation, IκBα Degradation, and COX-2 Expression in Murine Macrophages

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ABSTRACT

Macrophages crosstalk with oxidized low-density lipoprotein (oxLDL), play a critical role in the initiation, progression, and subsequently stability of atherosclerotic plaques. Statins, inhibitors of HMG CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, reduce the expression of inflammatory proteins in addition to their lipid-lowering action. However, the effect and detailed anti-inflammation mechanisms of statins in macrophages induced by oxLDL remain unclearly. In the present study, we investigated the effect of atorvastatin on inflammatory response upon oxLDL stimulation in murine macrophages and analyzed the underlying mechanisms. Tumor necrosis factor (TNF)α and monocyte chemoattractant protein-1 (MCP-1) mRNA levels were assayed by real-time PCR. The expression of cyclooxygenases-2 (COX-2) was detected by real-time PCR and Western blotting. While mitogen-activated protein kinase (MAPK) phosphorylation and IκBα degradation were determined by Western blotting. Our results showed that exposure of RAW264.7 cells to oxLDL, substantially changed the morphology of the cells and increased TNFα and MCP-1 secretion. While pretreatment with atorvastatin resulted in a significant inhibition of oxLDL-induced morphological alteration and inflammatory cytokines expression in a dose-dependent fashion. Further investigation of the molecular mechanism revealed that oxLDL upregulated the transcription and protein expression of COX-2 in a time-dependent manner. Whereas, pretreatment with atorvastatin suppressed COX-2 expression, MAPK activation and IκBα degradation. Thus, we conclude that the anti-inflammatory effect of atorvastatin is mediated through the inhibition of proinflammatory COX-2. Furthermore, suppression of ERK phosphorylation and IκBα degradation is involved in this regulation. Our findings provide a novel evidence that statins suppress inflammatory response, exert its anti-atherogenic actions via against inflammation beyond cholesterol-lowing effect. J. Cell. Biochem. 113: 611–618, 2012.

KEY WORDS: ATORVASTATIN; COX-2; oxLDL; MACROPHAGES; ATHEROSCLEROSIS

Atherosclerosis is a chronic, inflammatory disease in which immune mechanisms interact with metabolic risk factors. Oxidized low-density lipoprotein (oxLDL) crosstalks with macrophages, both play a crucial role in the initiation, propagation, and activation of atherosclerosis [Hansson, 2005]. In this process, macrophages take up oxLDL through the scavenger receptor and ultimately turn into lipid-loaded foam cells. More importantly, they also release proinflammatory cytokines and chemokines, such as monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor (TNF)α, and interleukin-1β (IL-1β) [Kirii et al., 2003; Weber et al., 2004], which result in lesions that are unstable and prone to rupture. Thus, much attention has been focused on macrophages. To effectively prevent and treat atherosclerosis and subsequent cardiovascular events, targeting macrophages seems desirable.

Statins, inhibitors of HMG-CoA (3-hydroxy-3-methylglutary coenzyme A) reductase, could reduce cholesterol synthesis, which are generally used as a treatment of hyperlipidemia [Gotto, 2003]. However, recent reports have shown that statins have other beneficial effects on cardiovascular risk in addition to their lowing endogenous cholesterol [Rosenson and Tangney, 1998; Corsini, 2000]. These pleiotropic effects include the upregulation of the production of nitric oxide in endothelial cells (ECs), the improvement or restoration of endothelial function; suppression of proliferation and migration of smooth muscle cells; inhibition of

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platelet activation and increase fibrinolytic activity [Palinski and Napoli, 2002; Liao, 2005; Kiyan et al., 2007]. Importantly, they could reduce both the proliferation and the activity of inflammatory cells within atherosclerotic lesions which exhibit the regulation in inflammatory process in the vascular system and the stabilization of atherosclerotic plaques [Koh, 2000]. Thus, statins may interfere with diverse biological pathways, which are involved in cell proliferation, migration, oxidative stress, and inflammation [Liao and Laufs, 2005]. In most cases, they display similar biological activities. However, in different cell types in response to different stimulation, they may mediate distinct cellular function through interaction with its own downstream signal pathways including mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K) [Liang et al., 2006; Paumelle and Staels, 2007].

Cyclooxygenases (COX)-2, an important inflammatory mediator, is induced by various stimuli and is involved in many inflammatory reactions and various physiological processes. It has been described that COX-2 is presented in atherosclerotic plaques and is localized mainly in macrophages. Activation of COX-2 has been implicated in the expression of inflammatory cytokines, such as MCP-1 and TNFα, which are involved in the progression of atherosclerosis [Burleigha et al., 2005]. Selective inhibition of COX-2 suppressed atherosclerotic lesion formation in LDL receptor-deficient mice. These evidences suggest that COX-2 serves as an important inducer of atherosclerosis [Burleigh et al., 2002].

Although statins have anti-inflammatory properties, their effect on oxLDL-induced inflammatory gene expression and mechanism has not been well established. In the present study, we investigated the effect of atorvastatin on inflammatory response upon oxLDL stimulation in murine macrophage and analyzed the underlying mechanism. Our data have shown that atorvastatin inhibited oxLDL-induced macrophage activation of morphological changes and inflammatory cytokines release. These effects are mediated by suppressing proinflammtory COX-2 signal pathway, and that the inhibition of ERK phosphorylation and IκBα degradation is involved in this effect.

MATERIALS AND METHODS

MATERIALS

(1) Antibody: Rabbit polyclonal anti-COX-2, anti-ERK1/2, anti-phosphoERK1/2, anti-IκBα, anti-GAPDH, and anti-β-actin were obtained from Cell Signaling Technology Inc. (Beverly, MA).

(2) Other chemical agents: Dulbecco's Modified Eagle's Medium (DMEM) and fetal calf serum (FCS) were from Hyclone. PD98059 (PD) was obtained from Sigma. Atorvastatin (at) was provided by Pfizer Incorporated. Tri Reagent was purchased from Molecular Research Center Inc. All the other chemicals were obtained from commercial sources.

ISOLATION AND OXIDATIVE MODIFICATION OF LDL

LDL (density range 1.019–1.063 g/ml) was isolated from normal human plasma by sequential ultracentrifugation, and dialyzed against PBS at 4°C. The LDL protein concentration was determined by a modification of the Lowry method with bovine albumin as the standard. After isolation, LDL was oxidized with $CuSO_4$ at 37°C for 18 h. Then oxLDL were sterilized by filtration membrane and stored at 4°C as described in our previous research and others [Steinbrecher et al., 1984; Shen et al., 2008].

CELL CULTURE

Raw264.7 cells (murine macrophage cell line) were obtained from the American Type Culture Collection and maintained in DMEM containing penicillin (100 U/ml), 100 μg/ml of streptomycin, and 10% heat-inactivated FCS at 37°C and 5% CO_2.

RNA PREPARATION AND REAL-TIME RT-PCR ANALYSIS

RAW264.7 cells (5×10^5 cells/well in 12-well plates) were incubated with various concentrations of atorvastatin, combined with or without 40 μg/ml oxLDL treatment. The total cellular RNA was extracted with Tri Reagent according to the manufacturer's instructions. cDNA synthesis was carried out with 500 ng of total RNA that was primed with random (dT). Quantitative real-time PCR was performed using SYBR Green (Toyobo) master mix and specific primers for mouse MCP-1, TNFα, and COX-2, which were designed as follows: MCP-1: forward primer, 5′-catccacgtgttggctca-3′ and reverse primer, 5′-gatcatcttgctggtgaatgagt-3′; TNFα: forward primer, 5′-gtccccaaagggatgagaagttc -3′ and reverse primer, 5′-tccacttggtggtttgctacgac-3′; COX-2: forward primer, 5′-cttcacgcatcagttttttcaag-3′ and reverse primer, 5′-tcaccgtaaatatgatttaagtccac-3′; GAPDH: forward primer, 5′- cccatgtttgtgatgggtgtg-3′ and reverse primer, 5′-tggcatggactgtggtcatga-3′.

The PCR conditions were as follows: preliminary denaturation at 50°C for 2 min; 95°C for 10 s, 95°C for 15 s, and 60°C for 1 min (40 cycles). The real-time PCR data were normalized by the levels of GAPDH mRNA and analyzed using ABI7900 Data Analysis software.

WESTERN BLOTTING ANALYSIS

The cells were seeded (1×10^6 cells/well) onto a six-well plate and lysed in a lysis buffer containing 150 mM of NaCl, 10 mM of Tris (pH 7.5), 5 mM of EDTA, 1% Triton X-100, 1 mM of PMSF, 10 mg/ml of leupeptin, 10 mg/ml of pepstatin, and 10 mg/ml of aprotinin for 30 min on ice. The protein concentrations were determined by the Micro BCA Protein Assay Reagent (Pierce). The lysates (50 μg) were electrophoresed on 10% SDS–PAGE and transferred onto the nitrocellulose membranes (Bio-Rad). The membranes were blocked with 5% (w/v) nonfat dried milk in TBST (50 mmol/L of Tris–HCl (pH 7.4), 150 mmol/L of NaCl, 0.1% Tween20) for 1 h and then incubated with various primary antibodies at a dilution of 1:1,000 in TBST, at 4°C overnight. The membranes were washed thrice with TBST and then incubated for 2 h at room temperature in TBST containing HRP-linked goat anti-rabbit or anti-mouse antibodies (Santa Cruz Biotechnology). Antigen detection was performed with the enhanced chemiluminescence kit (Millipore).

STATISTICAL ANALYSIS

The data were expressed as mean ± SEM. The statistical significance of the differences was analyzed using paired Student's *t*-test. A value of $P < 0.05$ was considered significant.

RESULTS

ATORVASTATIN ATTENUATES MORPHOLOGICAL CHANGES IN RAW264.7 CELLS INDUCED BY oxLDL

oxLDL has been proposed to be a key factor in the initiation and progression of atherosclerosis. Macrophage activation is triggered by inflammatory mediators or cytokines, which is associated with morphological changes [Lee et al., 2008; Ahn et al., 2009]. Recently, it has been found that statins, inhibitors of HMG-CoA reductase, have pleiotropic effects beyond their lipid-lowering action. First, we evaluated the effect of atorvastatin on morphological changes of macrophages induced by oxLDL. After oxLDL treatment, the cells showed morphological transformation including ruffle of the cell surface and exhibited multiangular, spindle shape. While pretreatment with atorvastatin suppressed the morphological alteration of these cells which retained their round shape (Fig. 1). Thus, atorvastatin blocked oxLDL-induced morphological changes, which indicate that it regulated the activation of macrophages in response to oxLDL.

ATORVASTATIN SUPPRESSES PROINFLAMMATORY CYTOKINE AND CHEMOKINE EXPRESSION IN RESPONSE TO oxLDL

Next, we tested the mRNA expression of proinflammatory cytokine and chemokine such as MCP-1 and TNFα in oxLDL-induced macrophages. Exposure of RAW264.7 cells to oxLDL resulted in the upregulation of MCP-1 and TNFα mRNA expression approximately to 10-fold, 14-fold, respectively, as compared with untreated cells. While preincubation with the indicated doses of atorvastatin, significantly reduced oxLDL-induced the mRNA expression of MCP-1 and TNFα in a dose-dependent manner, as assayed by real-time PCR (Fig. 2A and B, $P < 0.05$). These findings further suggest the involvement of atorvastatin in the regulation of oxLDL-induced macrophage activation.

UPREGULATION OF COX-2 IN oxLDL-INDUCED RAW264.7 CELLS

As known, COX-2, an important inflammatory mediator, is induced by various stimuli and is involved in many inflammatory reactions. First, we detected COX-2 expression in response to oxLDL. 40 μg/ml of oxLDL was chosen based on our preliminary experiment and previously published reports of others [Taketa et al., 2008]. When Raw264.7 cells were stimulated with oxLDL, both the mRNA and protein expressions of COX-2 were upregulated in a time-dependent manner. oxLDL increased COX-2 mRNA level by approximately 40-folds in 3 h, while COX-2 protein level was increased in 6 h, and remained up to 24 h (Fig. 3A and B, $P < 0.05$). The results suggest that oxLDL significantly stimulated COX-2 expression.

ATORVASTATIN REPRESSES COX-2 EXPRESSION UPON oxLDL STIMULATION

To further determine the mechanism involved in regulating inflammation in ox-LDL induced macrophages by statins, we investigated whether statins affect the oxLDL-induced expression of COX-2. RAW264.7 cells were incubated with various concentrations of atorvastatin in the absence or in the presence of 40 μg/ml oxLDL. In cells, treated with varying doses of atorvastatin (0, 10, 20, 40, 80, to 100 μmol/L) alone, did not modify COX-2 expression. While

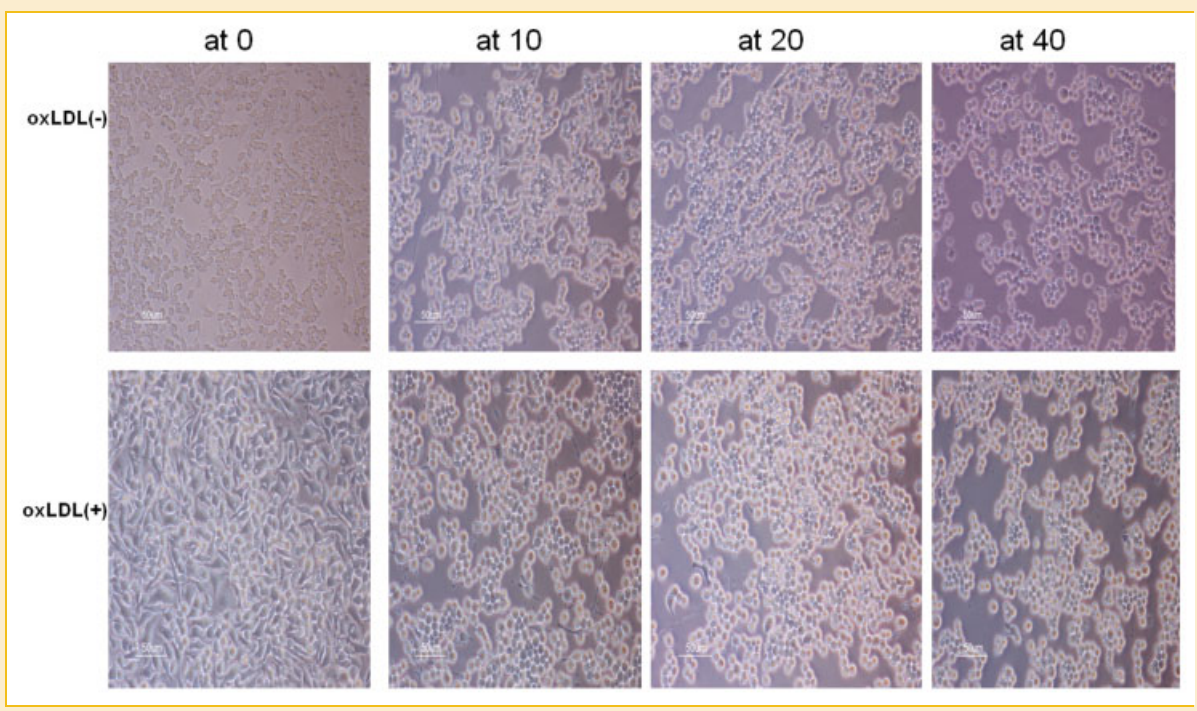

Fig. 1. Effect of atorvastatin on morphological changes induced by oxLDL in macrophages After pretreatment with atorvastatin in varying doses 0,10,20,40 μmol/L for 1 h, RAW264.7 cells were incubated with or without 40 μg/ml of oxLDL for 24 h. The images of the cells in culture were obtained using an inverted phase contrast microscope attached to a video camera.

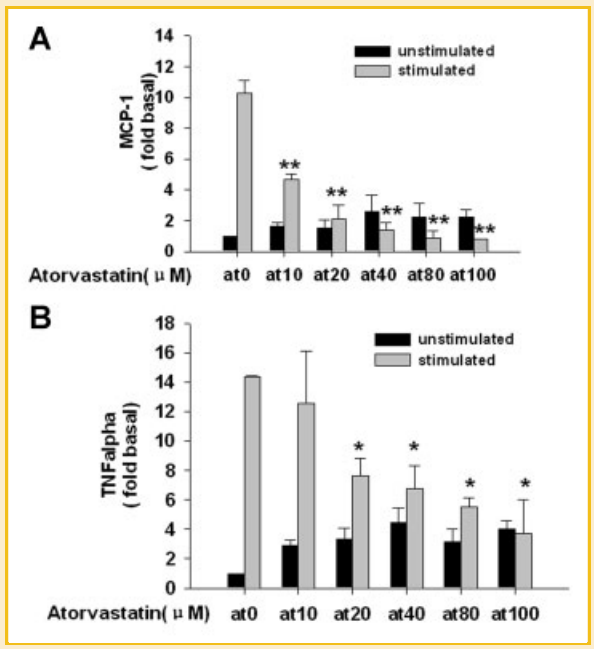

Fig. 2. Atorvastatin suppresses oxLDL-stimulated proinflammatory cytokine and chemokine expression The cells were preincubated with atorvastatin for different concentrations (0, 10, 20, 40, 80, 100 μmol/L) for 1 h, then in the absence or presence of oxLDL for 24 h. mRNA expression of MCP-1 (A) and TNFα (B) was determined by real-time PCR. at, atorvastatin $^{*}P < 0.05$, $^{**}P < 0.01$ versus cells incubated with oxLDL alone. Data are expressed as mean ± SEM in three independent experiments.

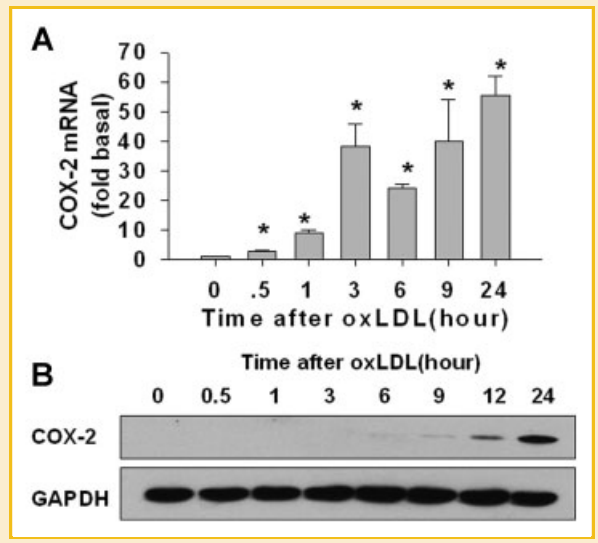

Fig. 3. oxLDL induces COX-2 expression (A) Raw264.7 cells were incubated with 40 μg/ml of oxLDL for the indicated times, and the expression of COX-2 mRNA was evaluated using real-time PCR. Data are expressed as fold difference from control in three independent experiments and are shown as mean ± SEM. $^{*}P < 0.05$ versus untreated control (time at 0 h). B: The expression of COX-2 protein was determined by Western blotting, and the GAPDH protein was monitored as a control. Experiments were repeated three times.

macrophages were pretreated with atorvastatin for 1 h, and then the cells were incubated with or without 40 μg/ml of oxLDL for 1 h. Total RNA was extracted and mRNA expression of COX-2 was determined by real-time PCR. As shown, exposure of cells to oxLDL resulted in the upregulation of COX-2. However, atorvastatin significantly inhibited mRNA expression of COX-2 in a concentration-dependent manner (Fig. 4A, $P < 0.05$). Likewise, COX-2 protein expression was upregulated in response to oxLDL, while addition with atorvastatin suppressed oxLDL-mediated COX-2 expression in a dose-dependent manner, as determined by Western blotting analysis (Fig. 4B). The results indicate that atorvastatin repressed mRNA and protein expression of COX-2 upon oxLDL stimulation.

ATORVASTATIN INHIBITS ERK PHOSPHORYLATION

To further explore the involvement of signal pathway in ox-LDL induced macrophages by statins, we investigated the MAPKs signal pathway which play an important role in cell proliferation, differentiation and production of several inflammatory genes [Tsatsanis et al., 2006]. First, we explored the potential role of atorvastatin on ERK1/2 phosphorylation in murine macrophages. As the results shown, the incubation with oxLDL for 1 h led to a high level of ERK1/2 phosphorylation compared with basal line (atorvastatin treatment alone). When given the pretreatment with different concentrations of atorvastatin, the oxLDL-induced phosphor-ERK1/2 activation was significantly decreased (Fig. 5A). Moreover, this effect is in a dose-dependent fashion.

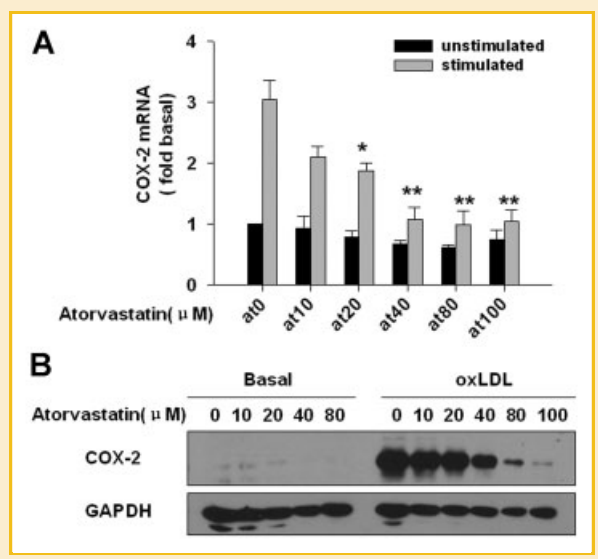

Fig. 4. Atorvastatin represses COX-2 expression upon oxLDL stimulation. A: Macrophages were pretreated with atorvastatin in varying doses 0, 10, 20, 40, 80, 100 μmol/L for 1 h, and then the cells were incubated with or without 40 μg/ml of oxLDL for 1 h. Total RNA was extracted and mRNA expression of COX-2 or GAPDH was determined by real-time PCR. at, atorvastatin $^{*}P < 0.05$, $^{**}P < 0.01$ versus cells incubated with oxLDL alone. Data are expressed as mean ± SEM from control in three independent experiments. B: Cells were preincubated with atorvastatin, or then exposed to oxLDL for 24 h, the whole cell extracts were prepared and probed with antibody COX-2, GAPDH expression served as controls for similar loading of proteins in each lane. The data represent three independent experiments.

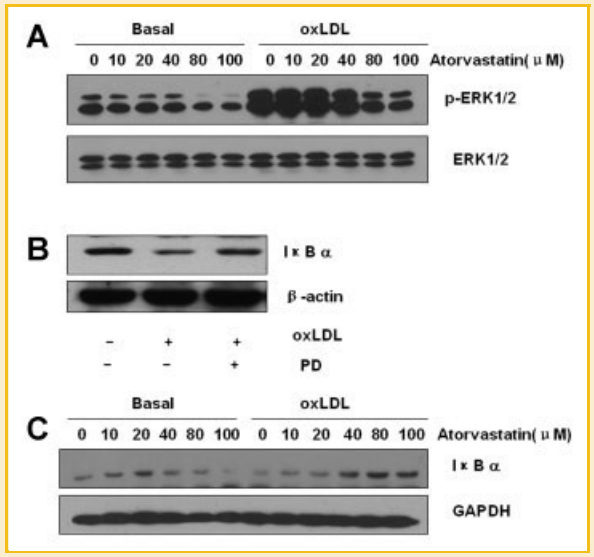

Fig. 5. Atorvastatin inhibits ERK phosphorylation and IκBα degradation in response to oxLDL (A) Macrophages were preincubated with atorvastatin at increasing concentrations (0, 10, 20, 40, 80, 100 μmol/L) for 1 h, then were incubated in the absence or presence of 40 μg/ml oxLDL for 1 h. Protein samples were immunoblotted for phosphorylated ERK1/2. Total ERK1/2 was used as an internal control. B: After preincubation with 20 μmol/L PD98059 (PD) for 1 h, cells were incubated with 40 μg/ml oxLDL for 1 h. The protein expression of IκBα was determined by Western blotting, the actin protein was monitored as a control, and similar results were obtained. C: Cells were pretreated with different concentrations of atorvastatin for 1 h and then with oxLDL for 1 h. Protein samples were prepared for Western blotting using anti-IκBα antibody. GAPDH expression served as an internal control. Experiments were repeated three times.

ATORVASTATIN SUPPRESSES IκBα DEGRADATION

In response to stimulation, IκBα, an inhibitor of NFκB, is phosphorylated, ubiquitinated, rapidly degraded via 26s protesome and released NFκB by IκBα kinase complex. Free NFκB is one of the key regulators of gene expression in the vessel wall and plays an important role in vascular inflammation. After degradation of IκBα, NFκB translocates to the nuclear and subsequently regulates the mRNA synthesis of target genes [Henkel et al., 1993; Baeuerle and Henkel, 1998]. IκBα protein expression was reduced following 40 μg/ml oxLDL treatment within 15 min, then quickly recovered to normal level at 3 h (data not shown). Next, we detected the effect of ERK1/2 specific inhibitor PD98059 on oxLDL induced IκBα expression. After oxLDL stimulation, IκBα protein expression was decreased, but remained the same as inactivation when cells were preincubated with 20 μM PD98059 (Fig. 5B). It revealed that the inhibitor blocked degradation of IκBα, suggesting the expression of IκBα is mediated by ERK1/2 signal pathway. Moreover, we further determined whether atorvastatin affect on degradation of IκBα upon stimulation in RAW264.7 cells by Western blotting analysis. The effect was significantly suppressed by pretreatment with varying doses of atorvastatin. Furthermore, atorvastatin reduced oxLDL-induced IκBα degradation in a dose-dependent manner (Fig. 5C). Taken together, the results indicate that atorvastatin suppressed ERK activation and IκBα degradation in response to oxLDL.

ERK PATHWAY IS INVOLVED IN oxLDL-STIMULATED COX-2 EXPRESSION

To demonstrate whether the ERK pathway is involved in COX-2 expression, RAW264.7 cells were incubated with oxLDL in the presence of 20 μM PD98059 or 20 μM atorvastatin. As the data shown previously, in Raw264.7 cells, oxLDL increased COX-2 mRNA and protein expression (Fig. 3A and B). However, pretreatment with PD98059, a specific inhibitor of ERK, this increase was significantly blocked both in mRNA and protein level, compared with cells treated with oxLDL alone (Fig. 6A and B, $P < 0.05$), suggesting that the ERK pathway is involved in oxLDL-induced COX-2 expression. Likewise, as previous shown, COX-2 mRNA and protein levels were markedly inhibited when cells were preincubated with atorvastatin (Fig. 6A and B, $P < 0.05$). From the results above, it revealed that oxLDL-induced COX-2 expression is mediated by ERK pathway.

DISCUSSION

In the present study, we have shown that oxLDL significantly induced macrophage activation by morphological changes and

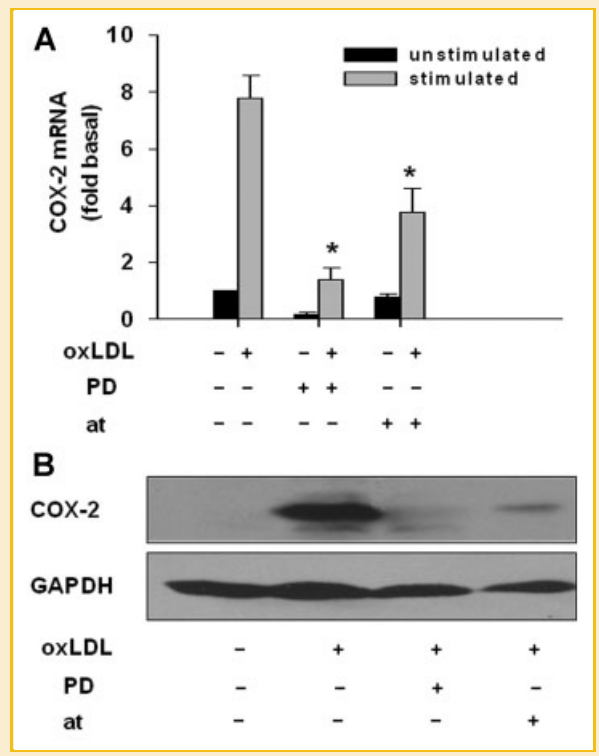

Fig. 6. oxLDL-induced ERK1/2 activation is involved in COX-2 expression (A) Raw264.7 cells were preincubated with 20 μM of PD or 20 μM of atorvastatin for 1 h, and then stimulated with 40 μg/ml of oxLDL for an additional 1 h. The mRNA was extracted to determine the COX-2 expression. PD, PD98059; at, atorvastatin $^{*}P < 0.05$ versus cells incubated with oxLDL alone. The data are presented as mean ± SEM of three separate experiments. B: After pretreatment with PD or atorvastatin for 1 h, cells were treated with in the absence or presence of oxLDL for 24 h. Whole cell extracts were prepared and performed to Western blotting analysis using anti-COX-2 antibody. Data were representative of three separate experiments.

inflammatory cytokines release. Further study indicated that oxLDL could also stimulate mRNA and protein expression of COX-2 in a time dependent manner. We also revealed that atorvastatin acts as a potent inhibitor of macrophage activation by blocking oxLDL-induced COX-2 expression in a dose-dependent manner, and that suppression of ERK phosphorylation and IκBα degradation is involved in this effect.

Atherosclerosis is not only a lipid metabolism disturbance, but also an inflammation condition of the vascular system. It seems essential to know how do the atherotic lesions initiate, progress, and most importantly, how do the lesions develop into vulnerable plaques which cause a serial clinical events. Apparently, a macrophage-oxLDL cross-talk plays a vital role in this process. Macrophages engulf oxLDL through the scavenger receptor pathways and transform into foam cells, which release proinflammatory cytokines, proteases, cytotoxic oxygen and nitrogen radical molecules. Ultimately, the oxidizing/inflammatory process promotes lesion progression and results in lesion unstable and prone to rupture [Hansson, 2005]. In our study, oxLDL, a well-known pro-inflammatory and pro-atherogenic stimuli, was used in murine macrophages. We demonstrated that oxLDL stimulation resulted in macrophage activation by morphological changes and inflammatory cytokines MCP-1 and TNFα upregulation.

Emerging data have shown that statins, inhibitors of cholesterol biosynthesis, in addition to lowing lipid action, have pleiotropic effects which affect vascular cells, inflammatory response and plaque stability [Takemoto and Liao, 2001]. Clinical trails have also shown they exert beneficial effects in the primary and secondary prevention of coronary artery disease [Laufs and Böhm, 2005]. Anti-inflammation property of statins may be due to the reduction of the release of proinflammatory cytokines. Recently, the effect of statins on vascular cells has been investigated. In endothelail cells, it has been reported that simvastatin and atorvastatin reduced COX-2 and MMP-9 expression and activity [Massaro et al., 2010]; Rosuvastatin also inhibited the expression of ICAM-1, MCP-1, IL-8, IL-6, COX-2, decreased monocyte-EC adhesion [Kim et al., 2007]. Moreover, atorvastatin reduced expression of COX-2 mRNA induced by IL-1β and TNFα in smooth muscle cells [Hernández-Presa et al., 2002]. Although some study suggest that the anti-inflammatory effects of statins are mediated through the inhibition of LPS-induced COX-2 and prostanoids in macrophages [Habib et al., 2007], Nareika et al. [2007] and Sundararaj et al. [2008] further revealed that simvastatin inhibited LPS-stimulated expression of IL-1β, TNFα, IL-6, and MMP-1 in U937 mononuclear cells. However, very little is known about the effect and the signal pathway of statins on inflammatory response induced by oxLDL in macrophages. In the current study, we found that atorvastatin attenuated morphological alteration and the upregulation of MCP-1 and TNFα in oxLDL activated macrophages, which exhibit anti-inflammatory properties.

Whereas, in response to various stimulation, statins exert distinct biological function through mediation, its own down stream signal pathways in different cell types. To better know the underlying mechanisms and the signal pathway involved in anti-inflammatory effect, first we explored the COX-2 pathway. COX-2, an inducible enzyme, is expressed in response to various inflammatory stimulation which also plays an important role in the development of atherosclerosis [Dubois et al., 1998]. It is responsible for the formation of the different prostanoids and their related signaling such as PGE2, which produced much inflammatory cytokines and participated in vascular biology [McAdam et al., 1999]. Our previous study also revealed that COX-2 inhibition could block oxLDL induced inflammatory cytokines expression [Shao et al., 2010]. Since the present study was designed to explore the mechanism of the inhibitory effect of statins on macrophage inflammation, the macrophages were co-treated with atrovastatin in the presence or absence of oxLDL. Our findings have shown that COX-2 expression was induced in a time-dependent fashion in response to oxLDL, which is in agreement with the reports by others [Lee et al., 2008]. While pretreatment with varying doses of atrovastatin, mRNA and protein expression of COX-2 was reduced in a dose-dependent manner.

Ample evidence indicate that MAPK cascades are the major signaling pathway which regulate cell growth, migration, differentiation, and production of inflammatory gene [Guha and Mackman, 2001]. Therefore, we speculated that ERK1/2 signaling pathways might be involved in the regulation of oxLDL-induced COX-2 expression by atorvastatin. In our study, atorvastatin exhibited significant inhibition on oxLDL-induced ERK1/2 phosphorylation in a concentration dependent fashion. To further confirm the essential role of ERK1/2 is involved in COX-2 expression, the effect of ERK1/2 specific inhibition on COX-2 expression was investigated. PD98059, an inhibitor of ERK1/2, abrogated both mRNA and protein expression of COX-2 upon oxLDL stimulation in cultured macrophages, suggesting that the regulation of inflammation in response to oxLDL is mediated through ERK1/2 signal pathway.

Some evidence indicating that nuclear factor NF-κB, one of the inducible transcription factors, is associated and controlled by IκBα, which presents an inactive form in the cytoplasm. Upon stimulation, IκBα becomes phosphorylated, undergoes degradation, and allows NF-κB translocation to the nucleus, where it activates proinflammatory genes and participates in the inflammation response [May et al., 2000]. It seems the function and expression of IκBα play an important role in the regulation of NF-κB activity. In the present study, our results suggest that atorvastatin suppressed oxLDL-induced IκBα degradation in a dose-dependent manner. Thus, it revealed that atorvastatin may decrease the NF-κB activity which is tightly associated with several inflammatory gene expression by inhibition of IκBα degradation. We also found that ERK1/2 specific inhibitor PD98059 blocked degradation of IκBα upon oxLDL stimulation, which suggest that IκBα degradation is a downstream event following the activation of the ERK1/2 pathway. Thus, the detailed mechanism of oxLDL-induced inflammatory response and the role in regulating inflammation by atorvastatin is described as shown in Figure 7. It would be interesting to explore other pathways involved in regulating inflammation by atrovastatin in macrophages.

In summary, here we show that atorvastatin exerts anti-inflammatory effect on macrophages by attenuating expression of proinflammatory signal molecule COX-2. Furthermore, the mechanism is involved in the inhibition of oxLDL-induced ERK1/2 activation and IκBα degradation. Taken together, our findings may provide a better understanding of the molecular mechanism of the

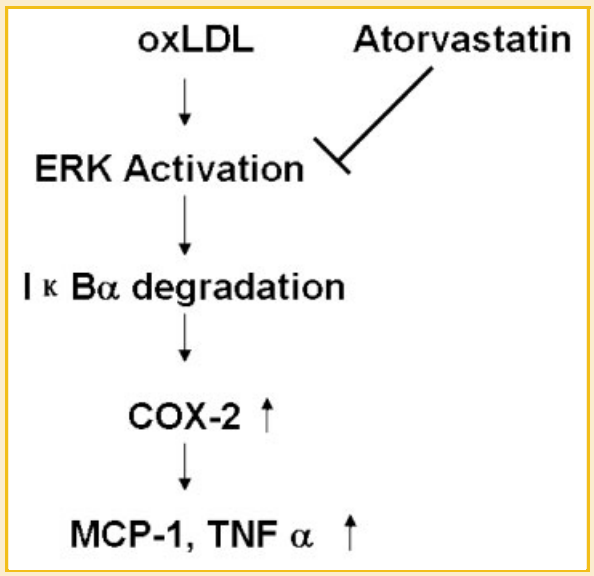

Fig. 7. The mechanism involved in the inhibition of oxLDL-induced inflammatory response by atorvastatin In this study, the results revealed the following scheme for the mechanism of atorvastatin inhibiting oxLDL-induced inflammatory cytokines release. When macrophages are treated with oxLDL, the expression of COX-2 and inflammatory cytokines were upregulated through ERK1/2 signal pathway. While, atorvastatin inhibits oxLDL-induced inflammatory response through reducing ERK phosphorylation, IκBα degradation, and COX-2 expression.

beneficial effects of statins therapy in cardiovascular disease. Thus, statins exert its anti-atherogenic actions via against inflammation beyond cholesterol-lowing action. Further investigation is needed to assess the role of statins on inflammatory response and the development of atherosclerotic progress in vivo.

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