Original Research

Lipoxin A4 attenuation of endothelial inflammation response mimicking pancreatitis-induced lung injury

Wanzhi Lv[1], Chongqing Lv[1], Suhui Yu[1], Yunxiu Yang[1], Hongru Kong[1], Jianming Xie[1], Hongwei Sun[1], Roland Andersson[2], Dan Xu[3], Bicheng Chen[1] and Mengtao Zhou[1]

[1]Department of Surgery, The First Affiliated Hospital, Wenzhou Medical College, Wenzhou 325000, Zhejiang Province, China; [2]Department of Surgery, Clinical Sciences Lund, Lund University and Lund University Hospital, SE-221 85 Lund, Sweden; [3]School of Optometry and Ophthalmology, Wenzhou Medical College, Wenzhou 325000, Zhejiang Province, China
Corresponding author: Mengtao Zhou. Email: zmt0417@hotmail.com

Abstract

Lipoxins (LXs) and their analogues are known to display potent anti-inflammatory actions. Previously, we reported that lipoxin A4 (LXA4) possessed powerful anti-inflammatory properties in acute pancreatitis in rats and that it may ameliorate the concomitant acute lung injury by reducing cytokine generation and inhibiting neutrophil activation. Considering that the vascular endothelium plays an important role during adherence, migration and activation of leukocytes, the present study was designed to investigate the effects of LXA4 on the inflammatory response induced by tumor necrosis factor α (TNF-α) in human pulmonary microvascular endothelial cells (HPMECs) and explore the potential mechanisms involved in these processes. We found that LXA4 markedly down-regulated the expression of monocyte chemotactic protein-1 (MCP-1), E-selectin, and interleukin-6 (IL-6) mRNA, as well as intercellular adhesion molecule-1 (ICAM-1) in TNF-α-exposed HPMECs. Moreover, LXA4 inhibited the phosphorylation and nuclear translocation of nuclear factor-κB/p65 (NF-κB/p65) and phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) in HPMECs following TNF-α stimulation. Heme oxygenase-1 (HO-1), a cytoprotective enzyme, was up-regulated by LXA4 in both non- and TNF-α-stimulated HPMECs. In conclusion, the protective effects of LXA4 to ALI may be executed through inhibition inflammation pathways of NF-κB and p38 MAPK and up-regulation of cytoprotective HO-1.

Keywords: Human pulmonary microvascular endothelial cells, lipoxin A4, tumor necrosis factor α, inflammation, nuclear factor-κB

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Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are the most common critical complications in severe acute pancreatitis (SAP).[1] Recently, there has been an increasing awareness that excessive inflammatory reaction plays a key role in SAP-induced ALI.[2,3] Systemic inflammatory cytokines can injure the lung endothelial cells and promote the expression of various pro-inflammatory mediators. Over-expression of adhesion molecules in injured endothelium, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin and E-selectin aggravate the inflammation through modulating the process of adherence, migration and activation of leukocytes.[4–6] Cytokines, adhesion molecules, and activation of leukocytes promote one another, ultimately leading to the progression of ALI and ARDS.[7]

Tumor necrosis factor α (TNF-α) is mostly derived from circulating monocytes and activated fixed-tissue macrophages in SAP. In the inflammatory cascade, TNF-α is considered as a 'proximal' mediator regulating the synthesis of various cytokines, chemokines, and adhesion molecules in endothelial cells and neutrophils.[2] For this reason, TNF-α can cause earlier endothelial injury and plays an important role in the occurrence of systemic complications and the development of ALI in SAP.[8]

Lipoxins (LXs) are arachidonic acid metabolites, which are endogenously produced locally and in later phases of multicelluar responses to, e.g. inflammation.[9] LXs and their analogues display potent anti-inflammatory effects, which are supported from multiple *in vivo* and *in vitro* studies.[10–13] Our previous data showed that LXA4, one of the LXs, possessed powerful anti-inflammatory properties and improved the SAP in rats. Ameliorated ALI in SAP was due to the LXA4 effects of reducing cytokine generation,

decreasing ICAM-1 expression, and inhibiting neutrophil activation.[14] However, the detailed mechanism of LXA4's protective effects on ALI remains unknown. Endothelial cells, as the main target of systemic cytokines in SAP, participate in cytokine secretion and leukocyte activation and transendothelial migration in ALI.[15] Whether LXA4 could modulate the expression of these key molecules in HPMECs *in vitro* is unclear. The present study aimed to investigate the effects of LXA4 on the inflammatory response of HPMECs stimulated by TNF-α and explore the potential mechanisms involved.

Materials and methods

Cell culture

HPMECs were purchased from ScienCell (San Diego, CA, USA) and maintained in PRMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 10% FBS (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin (Solarbio, Beijing, China) at 37°C humidified atmosphere with 5% CO_2. For the experiments, HPMECs were seeded onto 96-well plates, six-well plates or 75 mm culture dishes. The study was constructed with three groups: Control group (non-TNF-α stimulated HPMECs), TNF-α group (TNF-α-stimulated HPMECs), and LXA4 group (LXA4 pretreatment following TNF-α stimulation). Before each experiment, cells were grown in RPMI-1640 supplemented with 1% FBS for 12 h. The cells were in the initial experiments incubated under different concentrations (5 ng/ml, 25 ng/ml, or 50 ng/ml) of LXA4 (Cayman, Ann Arbor, MI, USA), resulting in a concentration of 50 ng/ml LXA4 to be used in the further experiments or a vehicle (0.035% ethanol) for 1 h before addition of 50 ng/ml TNF-α (Perprotech, Rocky Hill, NJ, USA). The effect of TNF-α on p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation was assessed at 0, 15, 30, and 60 min, and nuclear factor-κB/p65 (NF-κB/p65) translocation at 0, 30, 60, and 120 min. Furthermore, cells were pre-incubated with or without LXA4 for 1 h before stimulation with TNF-α for various periods: 4 h for measurement of mRNA levels of MCP-1, E-selectin and IL-6; 6 h for measurement of proteins expression of ICAM-1, and haeme oxygenase-1 (HO-1); 15 min for measurement of p38 MAPK phosphorylation, and NF-κB/p65 phosphorylation; 1 h for NF-κB/p65 translocation.

Cell viability assay

Cell viability was measured with cell counting kit-8 (cck-8) (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. Briefly, HPMECs were grown in 96-well plates and stimulated with TNF-α for 6 h in the presence or absence of the various concentrations of LXA4. At the set time point, the culture media was aspirated and fresh media supplemented with 10% (v/v) cck-8 was added to the cells. Cells were cultured at 37°C for 1 h and the plates were then read at a wavelength of 450 nm on an automated Tecan Sunrise absorbance reader. Cell viability was expressed as a percentage of the control culture value.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from HPMECs with TRIzol Reagent (Ambion, Carlsbad, CA, USA) following the manufacturer's instructions. Total RNA (1 μg) of each sample was subjected to oligo-dT-primed RT with ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). Real-time polymerase chain reaction (PCR) was performed for a quantitative analysis of monocyte chemotactic protein-1 (MCP-1), E-selectin and interleukin-6 (IL-6) mRNA expression using SYBR Green real-time PCR Master Mix (Toyobo) on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The following primers (Genecore, Shanghai, China) were used: 5-CTTCTGTGCCTGCTGCTCATAG-3 and 5-GCTTC TTTGGGACACTTGCTG-3, which amplifies the 170 bp product for MCP-1; 5-AGAGGTTCCTTCCTGCCAAGTG-3 and 5-AGAGCCATTGAGCGTCCATCC-3, which amplifies the 139 bp products for E-selectin; 5-GCCACTCACC TCTTCAGAACGA-3 and 5-CCTCTTTGCTGCTTTCACA CAT-3, which amplifies the 116 bp products for IL-6; 5-G TCTTCACCACCATGGAGAA-3 and 5-ATCCACAGTCTT CTGGGTGG-3, which amplifies the 267 bp product for GAPDH. Analysis of quantitative real-time PCR data was performed on ΔC_t values.

Protein extraction and Western blot analysis

For whole cell extraction, cells were lysed in radioimmune precipitation assay (RIPA) buffer supplemented with protease and phosphatase inhibitors (Roche, Basel, Switzerland). Nuclear and cytoplasmic proteins were extracted with nuclear and cytoplasmic extraction reagents (Beyotime, Jiangsu, China) according to the manufacturer's instructions. Equal amounts of proteins were separated on sodium dodecyl sulphate-polyacrylamide gels and transferred to nitrocellulose membranes (Solarbio). After being blocked with 5% nonfat dry milk, membranes were incubated overnight at 4°C with primary antibodies against p65, p- p65, ICAM-1, Lamin B (all 1:500, Bioworld Technology, Inc., Louis Park, MN, USA), p38, p-p38 (all 1:1000, Cell Signaling Technology, Inc., Danvers, MA, USA), HO-1 (1:500, Abcam Inc., Cambridge, MA, USA), GAPDH (1:5000, Bioworld Technology, Inc). Then, the membranes were washed three times with TBST and incubated with secondary antibodies for 1.5 h at room temperature. After washing 3 × 10 min with TBST, target bands were developed using ECL (Advansta, Menlo Park, California, USA) and exposed on a film. The intensity of protein bands was measured using Quantity One Version 4.6.2 Image software (Bio-Rad Laboratories, Hercules, CA, USA).

Immunofluorescence

HPMECs were cultured on sterile glass cover slips in six-well plates and treated with LXA4 and TNF-α as described above. Cells were fixed in 4% paraformaldehyde for 20 min at room temperature and washed with PBS. Cells were then permeabilized with 0.1% Triton X-100 in PBS. After washing with PBS, the cells were blocked with 5% goat serum for 1 h and incubated with rabbit anti-NF-κB/p65 (1:50, Bioworld Technology, Inc.) and anti-ALX (LXA4 receptor)/FRPL1 (1:50, Abcam Inc.)

antibodies overnight at 4°C. After washing, the cells were incubated with TRITC-conjugated goat anti-rabbit IgG (ZSJQ-BIO, Beijing, China) or FITC-conjugated goat anti-mouse IgG (ZSJQ-BIO) and nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI, Beyotime). After washing with PBS, the cover slips were mounted with anti-fade mounting medium (Beyotime) on the slides. Photography was performed using a fluorescent microscope (Leica Microsystems, Wetzlar, Germany).

Statistical analysis

Data are expressed as means ± SEM. Differences between mean values were analyzed by one-way analysis. Dunnett's *post hoc* test was used for multiple group comparisons. $P < 0.05$ was considered statistically significant.

Results

Expression of ALX in HPMECs

ALX/FRPL-1 receptor expression in HPMECs was marked using immunofluorescence analysis. In HPMECs, ALX is predominantly expressed in the cytosol in permeabilized cells (Figure 1).

Non-toxicity of LXA4 to HPMECs

The cytotoxicity experiments of LXA4 were performed at 5–50 ng/ml and the cell viability test showed that LXA4 is non-toxic to HPMECs at the dosages used in this study (data not shown).

LXA4 down-regulation of cytokines expression in TNF-α stimulated HPMECs

In order to study the effects of LXA4 on mRNA expression of MCP-1, E-selectin and IL-6 in TNF-α-induced HPMECs, quantitative real-time RT-PCR was performed. TNF-α significantly up-regulated the mRNA levels of MCP-1 (about 34-fold, $P < 0.01$), E-selectin (about five-fold, $P < 0.01$) and IL-6 (about five-fold, $P < 0.01$) as compared with controls in HPMECs at 4 h. However, the expression of MCP-1, E-selectin and IL-6 all decreased when cells were pretreated with various concentrations of LXA4 prior to TNF-α exposure. LXA4 inhibited TNF-α-stimulated up-regulation of E-selectin and IL-6 in a dose-dependent manner (Figure 2). MCP-1 was no different among pretreatment groups of various concentrations of LXA4.

LXA4 down-regulation of ICAM-1 expression in TNF-α stimulated HPMECs

ICAM-1 plays an important role during the whole course of SAP complicated by ALI, including the adhesion and activation of leukocytes.[16] The usage of NF-κB inhibitors has a positive effect on reducing the expression of ICAM-1 in the lungs, effectively reducing the extent of lung injury.[17] Therefore, we examined the effect of LXA4 on cell membrane expression of ICAM-1 in TNF-α-induced HPMECs. The Western blot analysis showed that pretreatment with LXA4 (25 ng/ml and 50 ng/ml) suppressed TNF-α-induced up-regulation of the ICAM-1 expression in HPMECs, whereas no difference was observed between the pretreatment groups. The presence of lower concentrations of LXA4 (5 ng/ml) was not significantly different compared with the TNF-α group (Figure 3a and 3c).

LXA4 inhibition of TNF-α induced NF-κB/p65 activation

Since phosphorylation and translocation of NF-κB play an important role in regulating the expression of pro-inflammatory cytokines genes, we want to investigate whether the down-regulation of LXA4 on TNF-α-induced pro-inflammatory cytokines is through suppression of the NF-κB pathway. Immunofluorescence analysis used to detect translocation of NF-κB/p65 showed that fluorescence strength increased significantly in the nucleus following TNF-α-stimulated HPMECs at 60 min, accompanied by the reduction of fluorescence strength in the cytoplasm (Figure 4a). However, pretreatment with LXA4 inhibited this translocation process (Figure 4b). To further verify the NF-κB/p65 nucleus translocation data, Western blot was performed to detect nucleus protein. We found that LXA4 markedly reduced the NF-κB/p65 level in nucleus in TNF-α-stimulated HPMECs (Figure 5a and b). In addition,

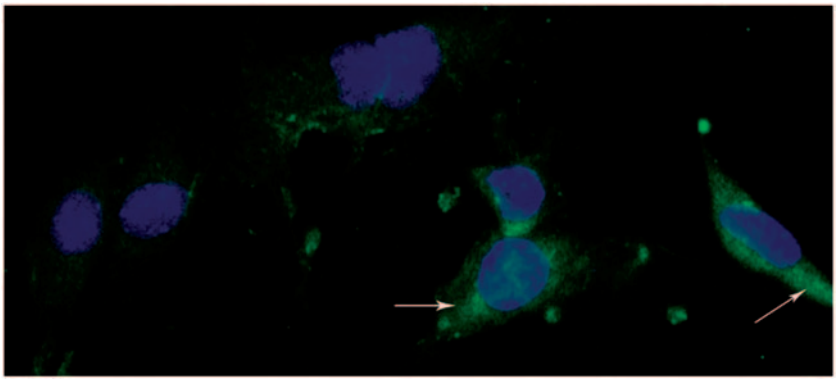

Figure 1 Expression of LXA4 receptor in HPMECs. HPMECs plated on sterile glass cover slips were fixed in 4% paraformaldehyde and were permeabilized with 0.1% Triton X-100 in PBS. The cells were stained with ALX Ab followed by FITC-conjugated goat anti-mouse Ab (green). Cells were counterstained with DAPI (blue). As arrows pointed out, ALX positively marked HPMECs was observed under fluorescent microscope, and photograph was recorded (×200). (A color version of this figure is available in the online journal)

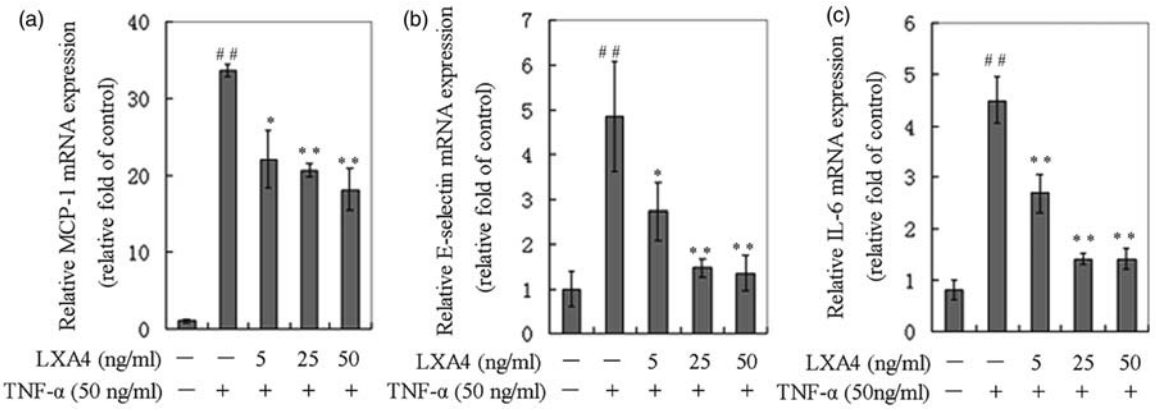

Figure 2 The effect of LXA4 on MCP-1, E-selectin, and IL-6 mRNA expression in TNF-α stimulated HPMECs. HPMECs were pretreated with LXA4 (5, 25 and 50 ng/ml) for 60 min followed by TNF-α stimulation (50 ng/ml). Total mRNA was prepared after 4 h stimulation, and expression of MCP-1 (a), E-selectin (b) and IL-6 (c) mRNA was measured by quantitative real-time PCR. After normalized by the controls, MCP-1, E-selectin and IL-6 mRNA were significantly decreased in LXA4 pretreated HPMECs. Each value represents the mean ± SEM for three independent experiments. $^{\#\#}P < 0.01$ compared with controls, $^{*}P < 0.05$ compared with TNF-α group, $^{**}P < 0.01$ compared with TNF-α group

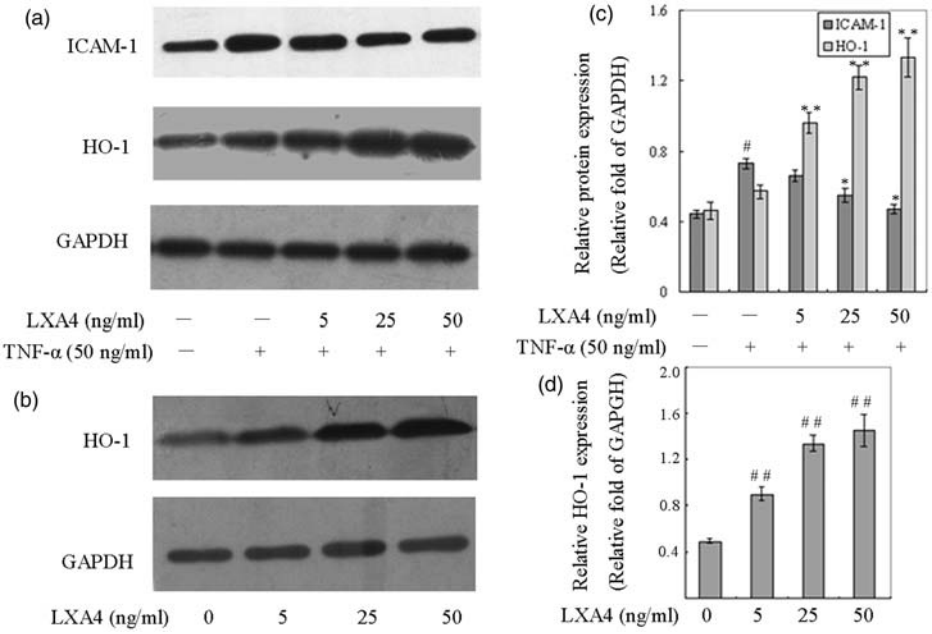

Figure 3 The effect of LXA4 on the expression of ICAM-1 and HO-1 in TNF-α stimulated HPMECs. HPMECs were pretreated with LXA4 (5, 25 and 50 ng/ml) for 60 min followed by TNF-α stimulation (50 ng/ml). The expression of ICAM-1 and HO-1 was determined by Western blot. After normalized to GAPDH, down-regulated ICAM-1 and up-regulated HO-1 were found in LXA4 pretreated HPMECs. Each value represents the mean ± SEM for three independent experiments. $^{\#}P < 0.05$ compared with controls, $^{\#\#}P < 0.01$ compared with controls, $^{*}P < 0.05$ compared with TNF-α group, $^{**}P < 0.01$ compared with TNF-α group

Western blot showed that the phosphorylation of NF-κB/p65 was increased significantly following TNF-α stimulation compared with control (0.78 vs. 1.50, $P < 0.01$) in HPMECs, whereas LXA4 suppressed TNF-α-induced phosphorylation of NF-κB/p65 (0.73 vs. 1.50, $P < 0.01$) in HPMECs (Figure 5a and c).

LXA4 inhibition the TNF-α induced p38 MAPK activation

The activation of p38 MAPK is known to induce expression of adhesion molecules and cytokines in human endothelial cells. To investigate whether the inhibition of inflammation by LXA4 was involved in the regulation of p38 MAPK pathways, we examined the phosphorylation of p38 MAPK in TNF-α-induced HPMECs using Western blot analysis. Previous studies have shown that TNF-α causes MAPKs phosphorylation within 15 min in human endothelial cells,[18,19] and our preliminary study is consistent with this (data not shown). Therefore, the 15-min treatment of TNF-α was determined to be optimal. As shown in Figure 6, TNF-α induced a marked increase in p38 phosphorylation compared with control (0.91 vs. 0.24, $P < 0.01$) in HPMECs. LXA4 reduced TNF-α-induced phosphorylation of p38 in

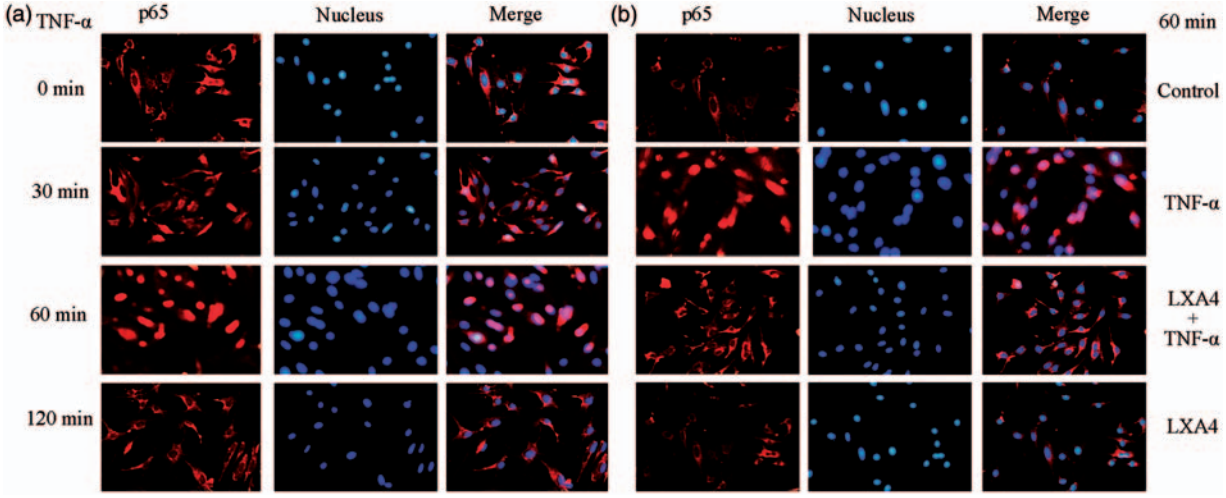

Figure 4 LXA4 inhibition of NF-κ B/p65 translocation in TNF-α-stimulated HPMECs. After TNF-α (50 ng/ml) stimulation for 0–120 min, subcellular location of the p65 in HPMECs was marked using Immunofluorescence assay. Among the time points, TNF-α stimulation for 60 min causes most significant translocation of p65 (×200, a). LXA4 pretreated (50 ng/ml, 1 h) HPMECs showed reduction of p65 translocation after TNF-α stimulation (50 ng/ml) for 60 min (×200, b). (A color version of this figure is available in the online journal)

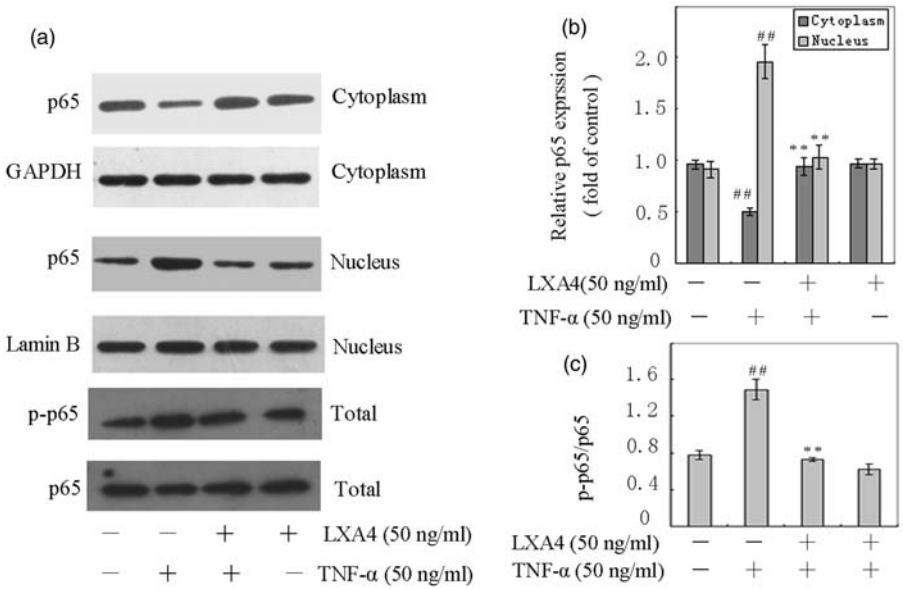

Figure 5 LXA4 inhibition of NF-κ B/p65 activation in TNF-α-stimulated HPMECs. LXA4 pretreated HPMECs (50 ng/ml, 1 h) was stimulated with TNF-α (50 ng/ml) for 60 min. Quantification of cytoplasmic and nuclear p65 bands was normalized by GAPDH or Lamin B, respectively. After TNF-α stimulation, p65 in cytoplasmic decrease and in nucleus increased. However, pretreatment with LXA4 inhibited this translocation process. HPMECs were pretreated with LXA4 (50 ng/ml) for 60 min, then stimulated with TNF-α (50 ng/ml) for another 15 min. Phosphorylation levels of p65 were analyzed using Western blot. Increased phosphorylation levels of p65 were significantly attenuated in LXA4 pretreatment group. Data are presented as mean ± SEM for three independent experiments. $^{\#\#}P < 0.01$ compared with controls, $^{**}P < 0.01$ compared with TNF-α group

HPMECs (0.36 vs. 0.91, $P < 0.01$). However, LXA4 did not influence the phosphorylation of p38 in non-TNF-α stimulated HPMECs ($P > 0.05$).

LXA4 up-regulation of HO-1 in HPMECs

Haeme oxygenase-1 (HO-1) activity is known to down-regulate inflammatory events and have cytoprotective effects. It has been shown that the aspirin-triggered lipoxin analog ATL-1 induces HO-1 expression on human umbilical vein endothelial cells (HUVEC).[20] To discern whether LXA4 regulates the expression of HO-1 in HPMECs, Western blot analysis was performed. As shown in Figure 3, the expression of HO-1 was markedly increased by LXA4 (5–50 ng/ml) as compared with control at 7 h. Further studies showed that LXA4-induced higher-level expression of

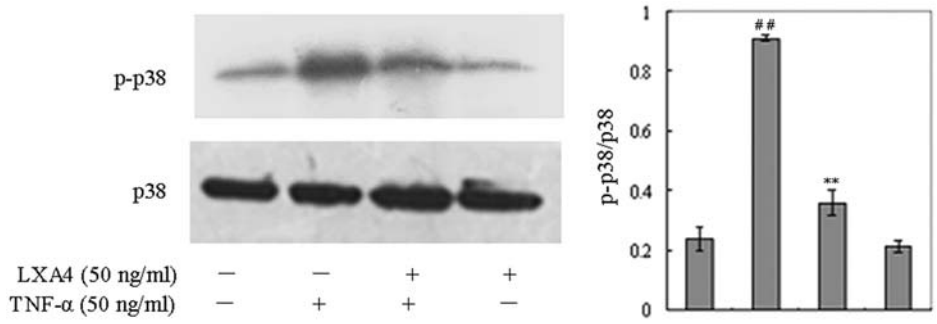

Figure 6 LXA4 inhibition of p38 MAPK activation in TNF-α-stimulated HPMECs. HPMECs was pretreated with LXA4 (50 ng/ml) for 1 h, then stimulated with TNF-α (50 ng/ml) for another 15 min. Phosphorylation of p38 was measured using Western blot. Data are presented as mean ± SEM for three independent experiments. $^{\#\#}P < 0.01$ compared with controls, $^{**}P < 0.01$ compared with TNF-α group

HO-1 still could be found in TNF-α-stimulated HPMECs (Figure 3).

Discussion

The pronounced systemic inflammatory response of SAP plays an important role in the development of ALI. The significantly increased inflammatory cytokines, such as TNF-α, cause the injury and activation of vascular endothelial cells, which contribute to the recruitment and activation of leukocytes.[2,3,8] Our previous study showed that LXA4 ameliorated SAP-induced ALI.[14] The protective effects may be due to inhibition effects of LXA4 to endothelial inflammatory response in lung. In the present study, we found that LXA4 down-regulated the expression of ICAM-1 and markedly decreased mRNA levels of MCP-1, E-selectin and IL-6 in HPMECs exposed to TNF-α. The NF-κB and p38 MAPK pathways as core signals involved in the inflammatory mechanisms in HPMECs were inhibited by LXA4. In addition, LXA4 significantly increased the expression of the cytoprotective enzyme HO-1 in both non- and TNF-α exposed HPMECs. Our data indicate that LXA4 attenuated TNF-α-induced inflammatory reactions of endothelial cells *in vitro*.

MCP-1, E-selectin, IL-6 and ICAM-1 are known to be important inflammatory mediators that contribute to the development of SAP and its induced ALI. Our results showed that LXA4 significantly inhibited the protein expression of ICAM-1 and the gene expression of MCP-1, E-selectin and IL-6 in HPMECs stimulated by TNF-α. These coincident results indicate the powerful anti-inflammation effects of LXA4 to HPMECs. Decreased MCP-1 down-regulates the chemotactic and activating effects on leukocytes, which will modify its signal and attenuate the trafficking of neutrophils and monocytes/macrophages invading the lungs in SAP.[21] LXA4 decreased TNF-α stimulated secretion of IL-6 in HPMECs, leading to changes of neutrophil deformability with a consequent increase in neutrophil sequestration in the lungs.[22–24] Membrane expression of ICAM-1 and E-selectin in HPMECs is another key factor involved in the adhesion, migration and activation of neutrophils.[6,16,17] Pretreatment with LXA4 should be beneficial to the interaction of HPMECs and neutrophils in inflammatory conditions. Consistent with our findings, previous results also showed that LXA4 inhibits pro-inflammatory cytokines in several other models of inflammation both *in vivo* and *in vitro*.[10,11,25,26]

NF-κB and MAPK are both essential pathways involved in regulating the gene expression of inflammatory mediators such as interleukins, chemokines, and adhesion molecules in endothelial cells exposed to TNF-α.[2,27] Activation of NF-κB pathway needs NF-κB phosphorylation and translocation from cell plasma to the nucleus, binding to the promoter region of various pro-inflammatory NF-κB responsive genes and activates transcription.[28,29] Our data showed that LXA4 markedly attenuated TNF-α-induced p65 phosphorylation and intranuclear translocation in HPMECs. LXs or their analogues induced reduction of nuclear translocation of NF-κB in neutrophils, mononuclear leukocytes, macrophages and microglial cells was reported in previous studies.[26,29–31] These findings suggest that the down-regulation of MCP-1, E-selectin and IL-6 by LXA4 in TNF-α-stimulated HPMECs may be via inhibition the NF-κB signaling pathway. In addition, we found that LXA4 can simultaneously inhibit p38 MAPK signaling pathway in HPMECs. This finding is consistent with studies in rat pulmonary microvascular endothelial cells,[26] neutrophils,[32] and microglial cells,[25] which have shown that p38 MAPK activation is attenuated in the presence of LXs or their analogues. These data evidenced that LXA4 has the universal effect of decreasing inflammation by inhibition of core inflammatory signal pathways. It has been verified that the p38 MAPK pathway is involved in the regulation of NF-κB activation in HUVECs;[33] however, the p38 MAPK pathway seems to have no effect on regulating the NF-κB pathway and regulating the expression of ICAM-1 and IL-8 at a post-transcriptional level in TNF-α-induced HPMECs.[18] The crosstalk of NF-κB and MAPK pathway following LXA4 intervention needs further study.

Our previous study demonstrated that LXA4 is beneficial to SAP-induced ALI. One potential explanation is that LXA4 has the property of cytoprotective effects to improve ALI. Numerous studies proved that HO-1 constitutes the major endogenous protective agent in organisms. It widely participates in various acute and chronic injuries, including inflammation-induced injury.[34–38] In the present study, LXA4 showed no cytotoxicity at treatment dosages. Moreover, induction of HO-1 expression by LXA4 showed a dose-dependently pattern, causing a persistent

up-regulation of HO-1 in TNF-α-induced HPMECs. This finding is in concordance with a previous study, which showed that the aspirin-triggered lipoxin analog ATL-1 induced expression of HO-1 in HUVEC in a concentration-dependent manner.[20] HO-1 synthesis triggered by ATL-1 is required for the inhibition of TNF-α-induced adhesion molecules expression.[20] HO-1 is the inducible isoform of the haeme oxygenase, which is able to modulate the inflammatory process. The results are supported by the study that HO-1 attenuated the activation of NF-κB pathway with strict requirement for the expression of pro-inflammatory cytokines.[39] Therefore, we suggest that HO-1-induction by LXA4 may have beneficial effects.

In conclusion, the present study demonstrates that LXA4 improved endothelial inflammation response mimicking SAP-induced lung injury. LXA4 down-regulated the expression of pro-inflammatory mediators in the TNF-α stimulated HPMECs. The anti-inflammatory effect of LXA4 may be mediated by modulating the NF-κB and p38 MAPK signaling pathways and up-regulation of HO-1 expression. These findings imply that LXA4 may be considered as a future potential therapeutic agent for the treatment of SAP-induced ALI or other inflammation-associated lung injury of different aetiologies.

Author contributions: WL, CL and SY performed the experiment. WL, YY, JX and HK analyzed the data. MZ, BC, HS and WL designed the experiments. WL and MZ wrote the paper. RA provided useful advice on design and reviewed the manuscript.

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