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Original Research Article

Modulation of epithelial sodium channel in human alveolar epithelial cells by lipoxin A4 through AhR-cAMP-dependent pathway

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Abstract

Purpose: *To investigate the effect of lipoxin A4 (LXA4) on the expressions of protein and mRNA of alveolar epithelial sodium channel (ENaC) in normal and lipopolysaccharide (LPS)-stimulated A549 cells.*
Methods: *A549 cell-lines were randomized into 11 groups (N = 8) and treated. EnaC level was evaluated by Western blot. Total RNA was extracted and reverse-transcribed and then levels of ENaC mRNA, cGMP and cAMP in the cells were determined.*
Results: *LXA4 (10^{-7}mol/L) increased the expressions of α-subunit of ENaC relative to LPS group. In addition, LXA4 significantly up-regulated the expression of mRNAs of α, β and γ subunits of ENaC ($p < 0.01$). The level of cAMP was increased in LXA4 group, but significantly reduced in LPS group relative to control group ($p < 0.05$). However, treatment with LXA4 annulled the increased cAMP concentration, compared with LPS group ($p < 0.05$)*
Conclusion: *These results show that LXA4 influences ENaC up-regulation in normal and LPS stimulated A549 alveolar epithelial cells.*

Keywords: *Acute lung injury, Alveolar epithelial sodium channel, Lipoxin A4, AhR, cAMP, cGMP*

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INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) lead to respiratory failure and pulmonary edema [1]. Alveolar epithelial injury is a major contributor to alveolar flooding. Injury to alveolar epithelium prevents re-absorption of edema fluid, a key step in the resolution of ALI/ARDS [1,2]. Earlier studies showed that alveolar epithelial sodium channel (ENaC) and Na-K-ATPase play critical roles in reducing edema in ALI/ARDS [3,4].

Lipoxins (LXs), as anti-inflammatory and pro-resolution mediators, have been extensively studied in various inflammatory diseases, as well as their interactions with lipoxin A_4 (LXA_4) receptors [5,6]. These receptors includde LXR (lipoxin receptor), $cysLT_1$ (cysteinyl leukotriene receptor 1), $cysLT_2$ (cysteinyl leukotriene receptor 2) and AhR (aromatic hydrocarbon receptor) [7]. Previous studies have demonstrated that post-treatment with LXA_4 significantly reduced ALI in LPS-stimulated mice [8]. A recent report showed that LXA_4 improved ENaC expression through ALX–cGMP signaling

pathways, resulting in enhanced clearance of alveolar fluid in oleic acid-stimulated animals [9].

The present study aims to test the hypothesis that LXA_4 increases ENaC protein and mRNA expressions in normal and LPS-stimulated A549 cells. In addition, it investigated LXA_4 receptors, ALX, AhR, $cysLRT_1$ and $cysLRT_2$ to determine the key receptors involved in up-regulation of ENaC function in A549 cells, as well as the levels of cAMP and cGMP in A549 cells.

EXPERIMENTAL

Materials

Lipopolysaccharide (LPS; *E. coil* serotype 055: B5) and AhR inhibitor, α-naphthoflavone (ANF) were purchased from Sigma (St. Louis, MO). Lipoxin A_4 was obtained from Cayman Chemical Company (Ann Arbor, MI). CysLT2 receptor inhibitor, BAY-u9773 was from Bimol Company (Farmingdale, NY). RPMI Medium 1640 and FCS were purchased from Gibco (Grand Island, NY). Saline citrate-buffered streptomycin and penicillin were products of Invitrogen (CA). The α, β and γ subunits of anti- ENaC were products of Abcam Company (Cambridge, MA), while anti-β-actin was supplied by Santa Cruz Company (Santa Cruz, CA). ELISA kits for cAMP and cGMP were purchased from R&D systems (Minneapolis, MN). Rp-cAMP (cAMP inhibitor) was obtained from Biomol-Enzo Life Sciences (Farmingdale, NY), while BCA protein assay kits and RT-PCR kits were purchased from Thermo Scientific (Rockford, IL). Real-time PCR Master Mix (SYBR Green) was obtained from Toyobo (Japan).

Cell culture and treatment

Human epithelial cell line (A549 cell line) was obtained from Basic Medical Sciences of Zhejiang Wenzhou Medical University. The cell line was cultured at 37 °C in a 5 % CO_2 humidified atmosphere, in RPMI 1640 medium containing 10 % fetal calf serum (FCS), 100 U/ml penicillin G and 100 µg/mL streptomycin. The cells were sub-cultured into six-well plates to 80 % sub-confluence; 100 % confluent cells were maintained for 24h in low serum medium (PRMI containing 0.1 % FCS). Thereafter, the cells were randomized into eleven groups (n = 8): control, LPS (1 µg/mL), LPS + ethanol (3.7 µl/mL), LPS + LXA_4 (10^{-9} M), LPS + LXA_4 (10^{-8} M), LPS + LXA_4 (10^{-7} M), LPS + LXA_4 (10^{-6} M), LXA_4 (10^{-7} M), ethanol, LXA_4 (10^{-7} M) + BAY-u9773 (3 µM) and LXA_4 (10^{-7} M) + ANF (1 nM) groups. In each group, the reagents were added simultaneously to stimulated cells and incubated for 6, 12 and 24 h. At the end of incubation, the cells were harvested and subjected to sonication.

Western blot for α, β and γ subunits of EnaC

Samples were lysed in standard RIPA lysis solution composed of 50 mM Tris buffer, pH 7.4, 150 mM NaCl, 1 % Triton X-100, 1 % sodium deoxycholate, 0.1 % SDS, 4 mM sodium pyrophosphate, 5 mM sodium fluoride, 1 mM EDTA, 0.02 mM leupeptin and 1mM phenylmethyl sulfonylfluoride (PMSF) on ice for 30 min, and thereafter centrifuged for 10 min at 12,000 rpm. The protein contents of the supernatants were estimated using BCA protein assay kitx. The protein mixture from the supernatants (80 µg) were separately boiled to achieve denaturation, and then subjected to 10 % SDS-PAGE prior to transfer onto PVDF membranes. Following blocking for 2 h with 5% non-fat dry milk in TBS-T buffer, expression of the α subunit of ENaC was determined using primary anti-epithelial sodium channel alpha antibody (1:750). The expression of the β subunit was determined using primary anti-SCNN1B antibody (1:500), while the expression of γ subunit was measured using primary anti-epithelial sodium channel gamma antibody (1:1000), and secondary goat anti-rabbit IgG. β-actin was used as internal control. Detection of the bound antibody was carried out on X-ray films by enhanced chemiluminescence (ECL).

RNA isolation, reverse transcriptase and PCR

Total RNA was extracted using Trizol reagent according to manufacturer's protocol. One (1) microgram of RNA sample was reverse-transcribed into complementary DNA (cDNA) using a RT-PCR kit on the MJ Mini Thermal Cycler (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. The primer sequences used for PCR amplification are in Table 1.

Then semi-quantitative analysis was performed by UVP-gel densitometry.

Quantitative real-time PCR

Levels of ENaC mRNA in A549 cells were determined with SYBR Prime Script Kit on the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Primers used for amplification are in Table 2.

Data was analyzed as previously described [9] using GAPDH as a reference gene.

Table 1: Primer sequence

Gene	Sequence length (bp)	Primer sequence
ALX	173	5'-TCCATTGTTGCCATCTGCTAT-3' (sense) 5'-AACATCTCT TTGAGCCAGACG-3'(antisense);
AhR	469	5'-TTAACGGATGAAATCCTGACG-3' (sense) 5'-AAGTAGTGGGGTATGGGGATG-3' (antisense)
CysLTR1	437	5'-GCCAGGTTTGTGTGTGTAGGT-3' (sense) 5'- GCAGCCAGAGACAAGGTTATG -3' (antisense);
CysLTR2	222	5'-TTGTGTTTCCTGCCCTATCAC-3'(sense) 5'-CTTTGTCTTTGCCTTCTGTGG-3' (antisense);
β-actin	204	5'-CCTTCCTGGGCATGGAGTCCT-3' (sense) 5'-GGAGCAATGATCTTGATCTT-3' (antisense).

Table 2: Primers for amplification

Variable	Sense	Antisense
α-ENaC	5'-CAGCCCATACCAGGTCTCAT-3'	5'- ATGGTGGTGTTGTTGCAGAA-3'
β-ENaC	5'- ATGGTGGTGTTGTTGCAGAA-3'	5'- CCAGGAAGGAGAAAACCACA-3'
γ-ENaC	5'- ACCACCAGCCATGGTCTAAG-3'	5'- GTTCAGGTCCCGGGATTTAT-3'
GAPDH	5'- GAGTCAACGGATTTGGTCGT-3'	5'-TTGATTTTGGAGGGATCTCG-3'

cAMP and cGMP assays

A549 cells were prepared, plated into six-well plates at a density 10^5/well and maintained sub-confluent (80 %). Confluent cells (100 %) were maintained for 24 h in serum-free medium, then treated with LPS (1 μg/ml), LXA_4 (10^{-7} M), BAY-u9773 (3 μM) or ANF (1 nM) for 24 h. The supernatants recovered after centrifugation were analyzed for contents of cAMP and cGMP by ELISA.

Statistical analysis

Data are expressed as mean ± SEM, and groups were compared by one-way ANOVA followed by Newman-Keuls multiple inter-group comparisons, using SPSS version 16.0 software. A probability of $p < 0.05$ was considered significant.

RESULTS

Dose-dependency of LXA_4 regulated ENaC expression in A549 cells stimulated with LPS

In all responses, only 10^{-7} M LXA_4 increased ENaC α subunit expression when compared with LPS group (Figure 1A). However, the expressions of the β and γ subunits of ENaC were increased dose-dependently, peaking at 10^{-7} M LXA_4 (Figure 1B and C). Therefore, in subsequent experiments, the expression of ENaC in A549 cells treated with LPS was assessed using 10^{-7} M LXA_4.

Time-dependency of LXA_4 regulated ENaC expression in A549 cells stimulated with LPS

At 6 h, LPS significantly decreased the expressions of α, β and γ subunits of ENaC when compared with control group ($p < 0.01$); while LXA_4 significantly increased the expressions of these subunits, compared with control group ($p < 0.01$; Figure 2). At 12 h, the protein levels of the α, β and γ subunits were decreased in the LPS group, but elevated in the LPS + LXA_4 group relative to the LPS group ($p < 0.05$; Figure 3). At 24 h, the levels of the α, β and γ subunits of ENaC were significantly decreased in the LPS group, but increased in the LPS + LXA_4 group when compared with the LPS group ($p < 0.01$; Figure 4). Therefore, in subsequent experiments, the effect of LXA_4 on ENaC expression in LPS-stimulated A549 cells was assessed at 24 h.

Effect of LXA_4 on the ENaC mRNA expression in A549 cells stimulated with LPS

The cells were incubated with LPS (1μg/mL) with or without LXA_4 (10^{-7} M) for 6 h. It was found that LXA_4 significantly up-regulated the expressions of mRNAs of α, β and γ subunits of ENaC ($p < 0.01$), and LPS decreased the mRNA level of ENaC α subunit relative to the control group ($p < 0.05$; Figure 5A). However, no significant change in mRNA expression of β and γ subunits was observed ($p > 0.05$; Figure 5B and C). Interestingly, the expressions of mRNAs of the β and γ subunits of ENaC were increased in LPS + LXA_4 group when compared with LPS group ($p < 0.05$), but not the ENaC α subunit.

Receptors of LXA_4 on A549 cells

As shown in Figure 6, LXA_4 receptor $cysLT_2$ and AhR mRNA were expressed in A549 cells. However, $cysLT_1$ and LXR mRNA were not expressed in A549 cells.

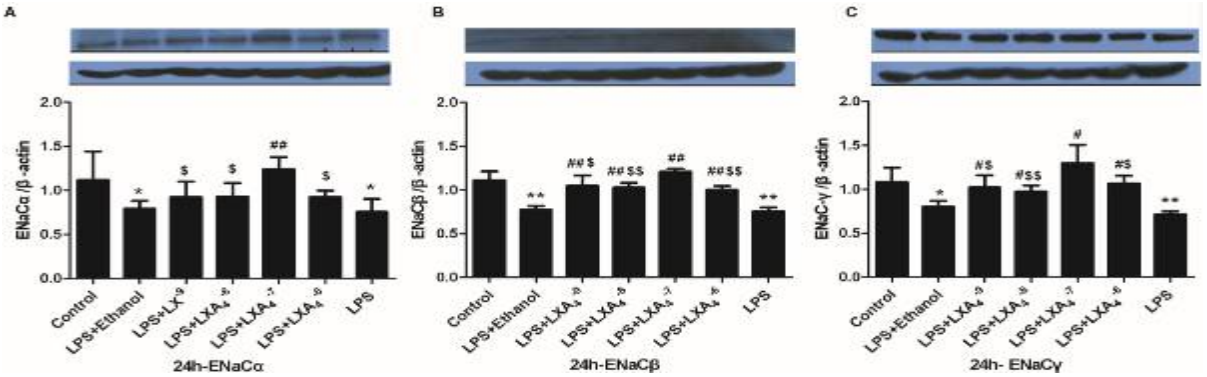

Figure 1: Effect of different concentrations of LXA_4 on the expressions of the α, β and γ subunits of ENaC after 24h in LPS-treated A549 cells. *A549 cells were treated with LXA_4 (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M) in the presence of LPS (1 µg/mL) for 24 h. Cells were then harvested, and sonicated. ENaCα (A), β (B) and γ (C) subunits protein were measured by western blotting. Results are presented as mean ± SEM for each group. *p < 0.05, **p < 0.01 compared to control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ relative to LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ compared to LPS + LXA_4 (10^{-7} M) group (n = 4). Ethanol was the resolvent*

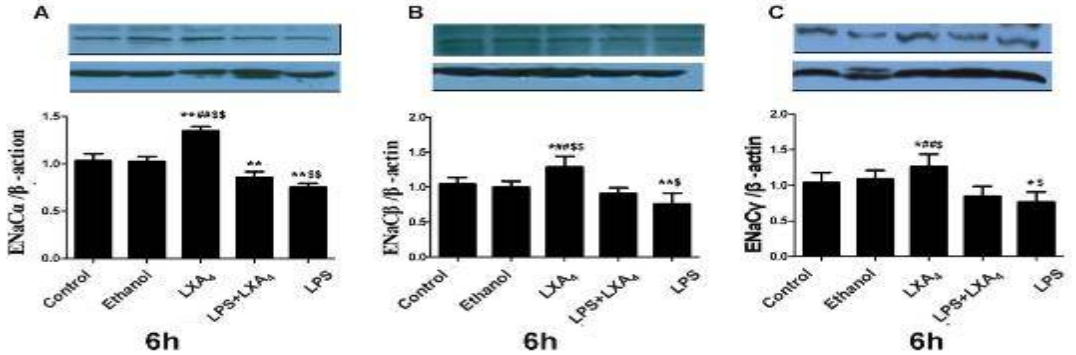

Figure 2: Effect of LXA_4 on ENaC α, β and γ subunits protein expression at 6 h in LPS-induced A549 cells. *A549 cells were treated with LXA_4 (10^{-7} M) in the presence or absence of LPS (1 µg/ml) for 6 h. Cells were then harvested, and sonicated. ENaCα (A, D, G), β (B, E, H) and γ (C, F, I) protein expressions at 6 h were determined by western blotting. Data are expressed as mean ± SEM for each group. *p < 0.05, **p < 0.01 versus Control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus Ethanol group (n = 4). Ethanol is resolvent*

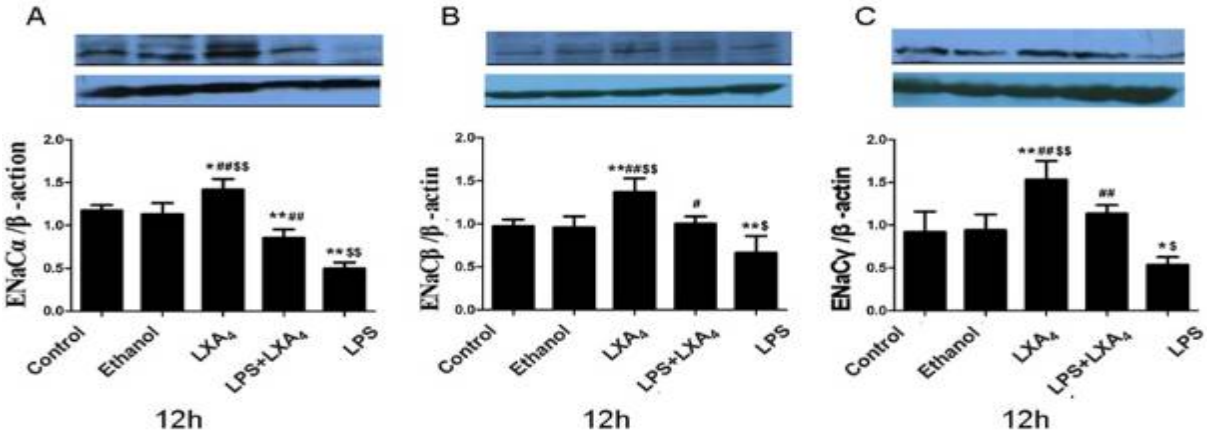

Figure 3: Effect of LXA_4 on ENaCα, β and γ subunits protein expression at 12 h in LPS-induced A549 cells. *A549 cells were treated with LXA_4 (10^{-7} M) in the presence or absence of LPS (1 µg/ml) for 12 h. Cells were then harvested, and sonicated. ENaCα (A, D, G), β (B, E, H) and γ (C, F, I) protein expressions at 12 h were determined by western blotting. Data are expressed as mean ± SEM for each group. *p < 0.05, **p < 0.01 versus Control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus Ethanol group (n = 4). Ethanol is resolvent*

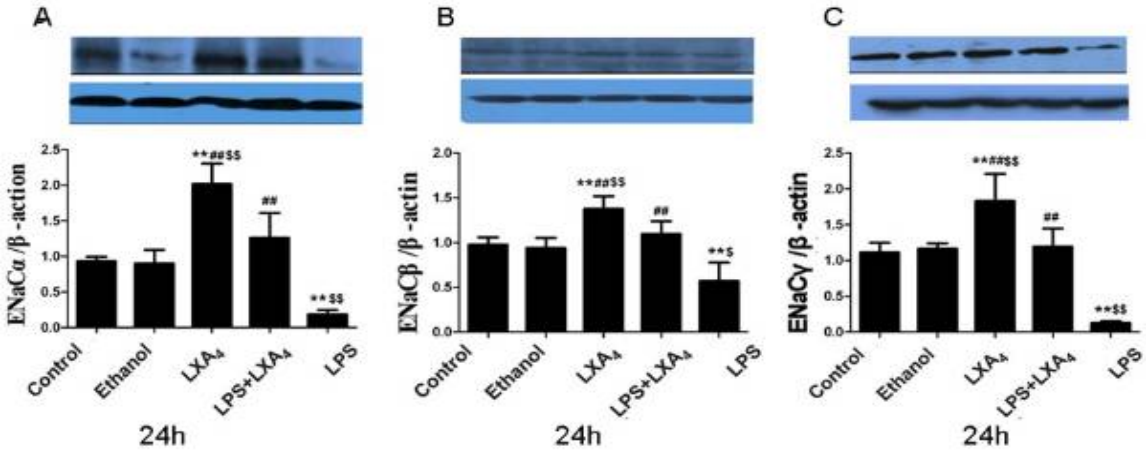

Figure 4: Effect of LXA_4 on expressions of α, β and γ subunits of ENaC at 24 h in LPS-induced A549 cells. *A549 cells were exposed to LXA_4 (10^{-7} M) in the presence or absence of LPS (1 μg/ml) for 24 h. The cells were then harvested, and sonicated. ENaCα (A, D, G), β (B, E, H) and γ (C, F, I) protein expression at 24 h were determined using western blotting. Data are expressed as mean ± SEM for each group. *p < 0.05, **p < 0.01 versus Control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus Ethanol group (n = 4); ethanol was resolvent*

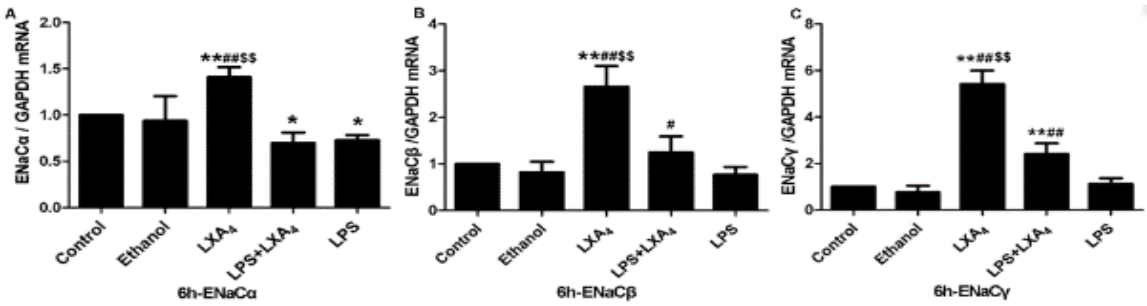

Figure 5: The effect of LXA_4 on the expressions of mRNAs of the α, β and γ subunits of ENaC at 6h in LPS-induced A549 cells. *A549 cells were treated with LXA_4 (10^{-7} M) in the presence and absence of LPS (1 μg/ml). After incubating for 6 h, the ENaC α (A), β (B), and γ (C) subunits mRNA expressions were determined by real-time PCR. Data are expressed as mean ± SEM for each group. *p < 0.05, **p < 0.01 versus control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus Ethanol group (n = 8). Ethanol was resolvent.*

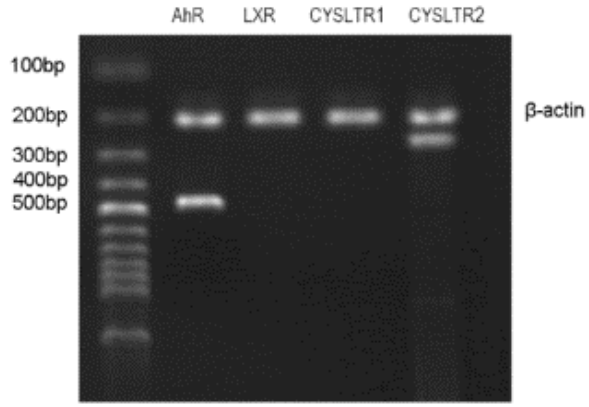

Figure 6: The expression of LXA_4 receptors in A549 cells. *A549 cells were treated with LXA_4 (10^{-7} M) for 24h. After incubation, the cells were harvested and sonicated. ALX (lipoxin receptor), AhR (aromatic hydrocarbon receptor), cysLRT1 (cysteinylLT1 receptor) and cysLRT$_2$ (cysteinylLT$_2$ receptor) were determined by reverse transcriptase-polymerase chain reaction (n = 8).*

Effect of LXA_4 receptors on expression of ENaC protein in A549 cells following LPS exposure

Protein expressions of the α、β and γ subunits of ENaC were up-regulated by LXA_4, but were blocked by the LXA_4 receptor inhibitor, ANF. However, BAY-u9773 was not inhibitory (Figure 7).

LXA_4 promoted ENaC expression

To test whether LXA_4 (10^{-7} M) had an impact on cAMP and cGMP levels in A549 cells, we measured cAMP and cGMP concentration in A549 cells exposed to LPS (1 μg/mL) for 24 h by ELISA kits. It was found that cAMP concentration was increased in the LXA_4 group, but was reduced in the LPS group relative to control group (p< 0.05). , and treatment with LXA_4 reversed the increased cAMP concentration when compared with the LPS group (p < 0.05; Figure 8A). In addition, when BAY-u9773 (cysLT$_2$

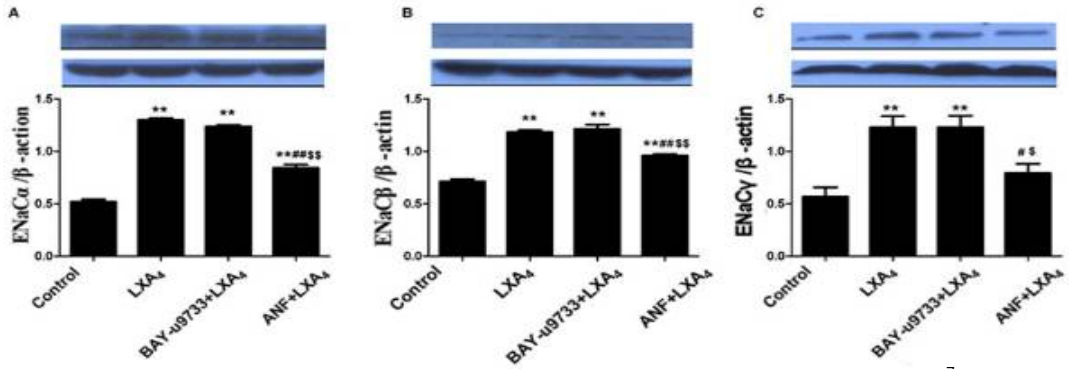

Figure 7: The effect of LXA_4 receptor antagonists on protein expression of ENaC in LXA_4 (10^{-7} M)-treated A549 cells. *A549 cells were exposed to LXA_4 for 24 h in the presence of either BAY-u9733 ($cysLRT_2$ antagonist, 3 μM) or ANF (AhR antagonist, 1 nM). After harvesting the cells and sonicating them, the protein expressions of the α (A), β (B) and γ (C) subunits of ENaC were determined by western blotting. Data are mean ± SEM. *p < 0.05, **p < 0.01 relative to control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ relative to LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus ethanol group (n = 4)*

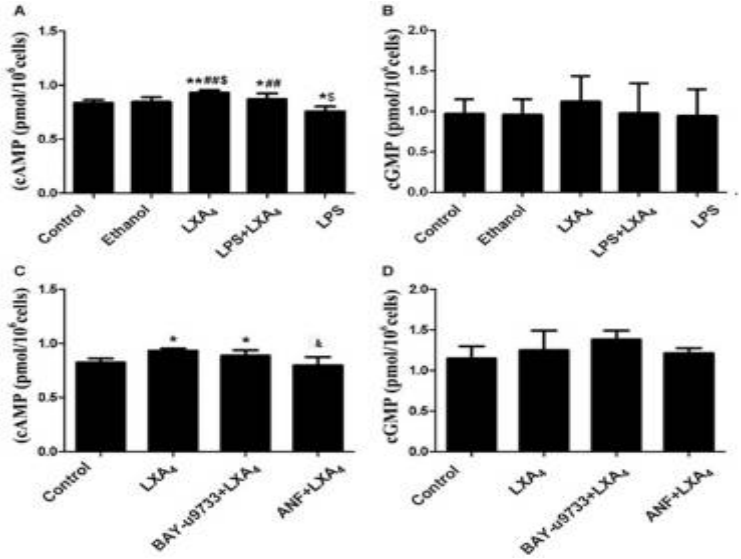

Figure 8: Effect of LXA_4 and LXA_4 receptor antagonists on cAMP and cGMP in A549 cells. *A549 cells were treated with LXA_4 (10^{-7}M) in the presence and absence of LPS (1 μg/mL), BAY-u9773 ($cysLRT_2$ antagonist, 3 μM) or ANF (AhR antagonist, 1 nM) for 24 h. cAMP (A) and cGMP (B) protein concentration of suspension were determined by ELISA kits. Data are expressed as mean ± SEM for each group. *p < 0.05, **p < 0.01 versus control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus LPS group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus Ethanol group, $^{\&}p < 0.05$ versus LXA_4 group (n = 8). Ethanol was resolvent*

antagonist, 3 μM) and ANF (AhR antagonist, 1nM) were used to treat A549 cells in the presence of LXA_4 for 24 h, cAMP concentration was decreased in the LXA_4 + ANF group relative to the LXA_4 group ($p < 0.05$), but not in the LXA_4 + BAY-u9773 group (Figure 8C). However, no significant change was seen in cGMP levels in these groups (Figure 8B & Figure 8D).

The protein levels of α, β and γ subunits were markedly decreased in LPS + LXA_4 + Rp-cAMP group when compared with LPS + LXA_4 group (Figure 9).

DISCUSSION

ALI/ARDS is a common, devastating clinical syndrome that affects large numbers of patients and has up to 40 % mortality [10]. The current treatment for ALI/ARDS is aimed at removal of polymorphonuclear neutrophils, and alveolar fluid re-absorption. Cation and anion channels and ion transporters are involved in the clearance of alveolar fluid. Several studies have reported the key role of ENAC not only in the uptake of Na+ from the alveolar fluid but also in the clearance of the fluid itself [2,3,11-13]. In the present study, it has been clearly demonstrated that LXA_4 up-regulated the expressions of the mRNAs and proteins of the α, β and γ subunits of ENAC in normal and LPS-treated human alveolar epithelial cells. These up-regulations were both dose- and time-dependent. These effects were blocked by the inhibitor of AhR and Rp-cAMP, an

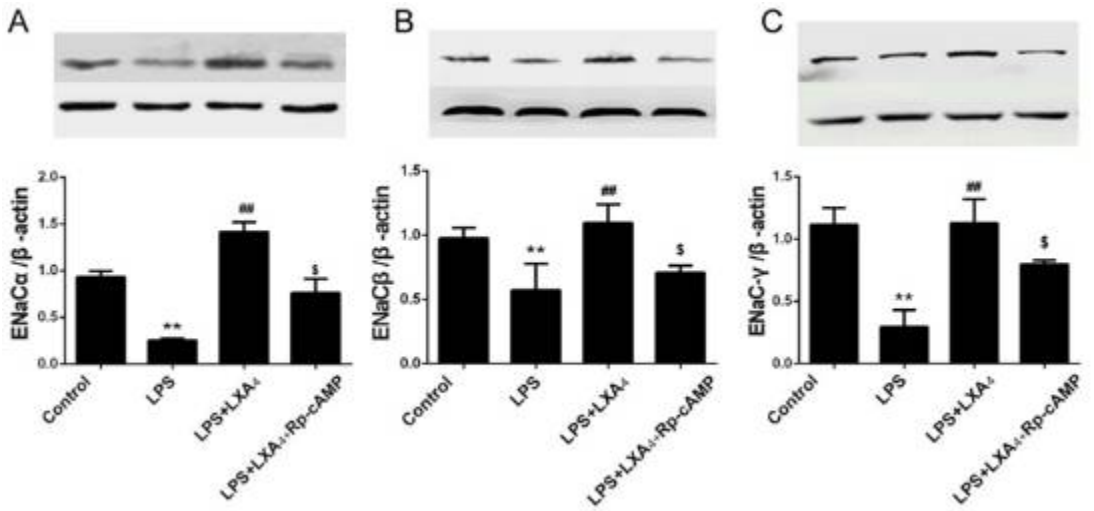

Figure 9: LXA_4 increased ENaC expression dependent on cAMP in A549 cells stimulated with LPS. *Cells were treated with RP-cAMP (cAMP inhibitor, 10 uM) in the presence of LXA_4 (10^{-7} M) and LPS (1 ug/mL) for 24 h. After incubation, the cells were harvested, and sonicated. ENaC α, β and γ subunits protein expression in the cell lysates were determined by western blotting using a specific antibody*

indication that LXA_4 enhanced the expression of ENaC through AhR-cAMP signaling pathway.

The expressions of the mRNAs and proteins of the α, β and γ subunits of ENAC were decreased by 1μg/mL LPS, but these decreases were dose-dependently reversed by LXA_4, with maximal effect at 10^{-7} M. These findings are in disagreement with recent reports which suggest that LXA_4 enhanced the expression of ENaC in A549 alveolar cells [10]. Interestingly, it was found that 10^{-6} M LXA_4 was less effective than 10^{-7} M. This implies that the effect of LXA_4 was not concentration-dependent. It is also likely that at 10^{-6} M, LXA_4 actually brought about decreased expression of ENaC. Thus, 10^{-6} M LXA_4 was used in subsequent experiments. LXA_4 significantly improved the expressions of the α, β and γ subunits of ENAC in healthy A549 cells and in LPS-treated cells at 6, 12 and 24 h. The expressions in the LPS group at 24 h were much lower than those at 6 h and 12 h, suggesting a greater degree of cellular damage at 24 h than at any of the other periods. It has been reported that LXA_4 increased ENaC concentrations in primary rat alveolar type II epithelial cells exposed to LPS 24 h [21]. Therefore, in subsequent studies, the effect of LXA_4 on ENaC expression in LPS-treated A549 cells was evaluated at 24 h. The results obtained in the present study show that LXA_4 regulates the expression of ENaC in healthy and pathological states. This is consistent with previous results which suggest that LXA_4 may be involved in endotoxin-induced ALI/ARDS [14].

The expression of the mRNA of the α subunit of ENAC was decreased following exposure to 1 μg/mL LPS for 6 h, but there were no significant changes in the expressions of mRNAs for the β and γ subunits. This shows that the expression of mRNA of the sodium channel was inhibited by LPS. In a previous study, it was reported that mouse alveolar epithelial cells treated with LPS for 8 h had significantly reduced expressions of mRNAs for the α, β and γ subunits of ENAC [27]. The fact that LXA_4 increased mRNA expressions for α, β and γ of ENAC at 6 h indicates that LXA_4 protected the cells by enhancing ENaC gene translation.

LXs mediate their effects by interacting with one or more specific receptors such as ALX, CysLTR1, CysLTR2 and AhR. In this study, CysLTR2 and AhR were the only receptors present on the basolateral membrane of the A549 cells, which is consistent with previous findings showing that A549 cells expressed mRNAs of $cysLT_2$ and AhR only [16-18]. The beneficial effects of LXA_4 were reversed by ANF, an AhR antagonist, but not by the $cysLT_2$ receptor antagonist BAY-u9773. This is clear evidence that the effect of LXA_4 on expression of the α, β and γ subunits of ENAC occurred through binding to AhR.

cAMP and cGMP are important second messengers by which cells transduce extracellular signals into intracellular responses. Extracellular signals interact with GPCRs to activate adenylate cyclase (AC) and increase intracellular cAMP levels. A previous study showed that LPS-induced immune responses are associated with decreases in intracellular cAMP levels [7]. It has been reported that LPS activates inhibitory G protein, thereby inhibiting formation of cAMP [19]. Stimulation by cAMP enhances Na^+ transport and the activity of Na-K-ATPase in

the plasma membrane [20, 21]. This is in agreement with a model that suggests that at the early stages, cAMP enhances the transport of ENaC to the cell surface, whereas the synthesis of ENaC is necessary for the sustenance of this effect [20]. Extracellular signals also interact with GPCR to activate guanylyl cyclases (GC) and increase intracellular cGMP levels. Studies have shown that AhR induces signaling pathways that entail ERK, PKA, MMP9 and cAMP, cGMP and Ca^{2+} [22]. It has also been shown that AhR is more sensitive to the cAMP signaling pathway [22,23], and that cAMP induces nuclear translocation of the AhR [24]. Thus it appears that the two signaling pathways for regulation of ENaC, cAMP, and cGMP are compart-mentalized. Levels of intracellular cAMP were decreased after LPS stimulation, and the decreases were reversed by LXA_4 in the LPS group. The reversal of the effects of LXA_4 by ANF and Rp-cAMP, without any significant effects on intracellular cGMP levels indicates that LXA_4 increases the levels of cAMP through AhR. Thus LXA_4 up-regulates the expressions of α, β and γ subunits of ENaC by activating cAMP via AhR. The role of LXA_4 in LPS-treated human alveolar epithelial cells can be depicted as indicated in Figure 10.

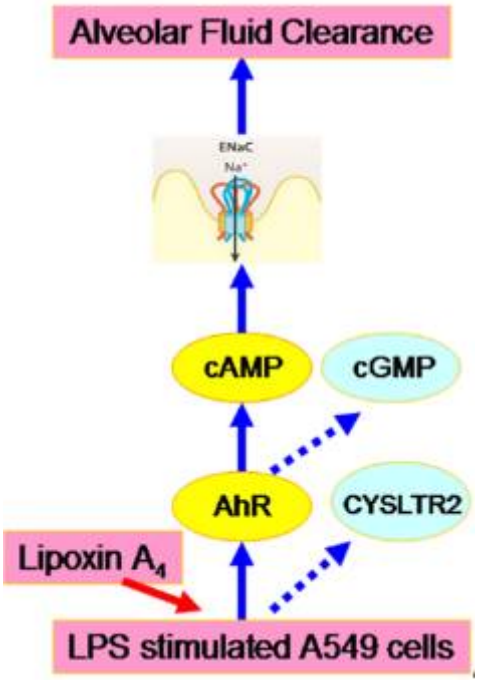

Figure 10: Modulation of sodium channel in human alveolar epithelial cells LXA_4 through AhR-cAMP-dependent pathway

CONCLUSION

This study has provided evidence for up-regulation of ENAC in normal and LP-stimulated A549 alveolar epithelial cells, most probably through the AhR–cAMP signaling pathways. Thus, it is proposed that LXA_4 can be applied for the induction of ENaC expression in the treatment of ALI/ARDS and related diseases.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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