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Lipoxin A4 mitigates experimental autoimmune myocarditis by regulating inflammatory response, NF-κB and PI3K/Akt signaling pathway in mice

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Abstract. – OBJECTIVE: Myocarditis, an acute inflammation disease of the heart, is a potentially lethal disease and can lead to sudden death. This study aims to investigate the therapeutic effect of lipoxin A4 (LXA4) on experimental autoimmune myocarditis (EAM) and to explore the underlying mechanism.

MATERIALS AND METHODS: EAM was induced in BALB/c mice by injection of porcine cardiac myosin and LAX4 at doses of 10 or 50 μg/kg was administrated from day 1 to 21. The severity of myocarditis was evaluated by detection of heart weight/body weight (HW/BW) ratio and histopathological examination of the heart. Cardiac function and heart structure were assessed by echocardiography. Serum levels of Th1 and Th2 cytokines were determined by ELISA. Protein expression was detected by Western blot analysis.

RESULTS: The results demonstrated that LXA4 mitigated the severity of myocarditis by decreasing HW/BW ratio and reducing infiltration of inflammatory cells. Echocardiographic analysis indicated that cardiac function of LXA4-treated rats was significantly improved compared with non-treated group. LXA4 treatment significantly increased the levels of Th1 cytokines (TNF-α and IL-6) and decreased Th2 cytokines (IL-4 and IL-10). Furthermore, LXA4 administration effectively inhibited NF-κB nuclear translocation and deactivated PI3K/Akt pathway.

CONCLUSIONS: LXA4 has a protective effect against EAM by reducing the inflammatory response and inhibiting NF-κB and PI3K/Akt signaling pathway.

Key Words:
Lipoxin A4, Experiment autoimmune myocarditis, Inflammation, NF–κB, PI3K/Akt.

Introduction

Myocarditis is defined as an inflammation of myocardium with consequent myocardial injury. It is a precursor of dilated cardiomyopathy (DCM) and represents the most common cause of chronic heart failure, or even sudden death[1,2]. Although the pathogenesis of myocarditis is not fully understood, there is considerable evidence indicating that autoimmunity may arise due to inefficient elimination of the pathogen resulting in overaggressive immune surveillance and/or due to antigenic mimicry between pathogenic epitopes and cardiac myosin[3,4]. In animals, infiltrating T lymphocytes and secreted cytokines have been shown to play prominent roles in the establishment of various autoimmune disease models such as experiment autoimmune myocarditis (EAM)[4]. EAM in rodent may be elicited by immunization of cardiac myosin, and EAM in rats mimics human fulminant myocarditis in the acute phase and human DCM in the chronic phase[5,6]. This animal model has been shown to be mediated by T cell and been particularly suitable for studying immunopathology of myocarditis. Up to now, there is no universally ac-

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cepted effective therapy for myocarditis. The treatment for myocarditis are directed toward reducing or eliminating the inciting agent when possible and tailoring therapy toward the associated complications such as congestive heart failure, dysrhythmias and thromboembolism[7]. Meanwhile, toxicity and limited efficacy are common disadvantages of the anti-inflammatory and/or immunosuppressive drugs. Hence, the identification of the new agents with low toxicity is very useful for the treatment of myocarditis and DCM. Lipoxins, a class of endogenous anti-inflammatory lipid-based autacoids, are generated from arachidonic acid (AA) via sequential actions of lipoxygenases during the onset of the inflammatory response[8,9]. Many studies[10] show that lipoxygenase enzymes such as 12/15 Lox can catalyze the conversion of AA into biologically active lipid mediators to regulate inflammatory responses. Also, a report[11] proves that AA can make p38, one important member of MAPK signal pathway phosphorylated to fight against systemic inflammatory reaction, shock, cell migration and cell apoptosis. Lipoxin A4 (LXA4) is the major physiological lipoxin during inflammation in mammalian systems[12]. Lipoxins, including LXA4 have been proven to demonstrate powerful anti-inflammatory function under many pathological conditions related to inflammation, including asthma[13], inflammatory pain[14], and arthritis[15]. Based on these beneficial effects of LXA4, this study aims to investigate the therapeutic effect on EAM and to explore the underlying mechanisms in mice.

Materials and Methods

Animals and Treatments

Six-week old BALB/c mice were obtained from the Experimental Animal Center of Suzhou Aiermaite Technology Co. Ltd. (SPF grade, Certificate No. SCXK20140007, Suzhou, China). All mice were housed in the animal care facility at the Laboratory Animal Center, Provincial Hospital Affiliated to Shandong University and provided food and water *ad lib*, under standard condition. All of the animal procedures were approved by the Institutional Authority for Laboratory Animal Care and were performed in accordance with the Guidelines for Animal Experiments of Provincial Hospital Affiliated to Shandong University.

Immunization

EAM was induced in BALB/c mice by subcutaneous injection of 0.1 ml of porcine cardiac myosin (6 mg/ml, Sigma-Aldrich, St. Louis, MO, USA), mixed with an equal volume of Freund's complete adjuvant (FCA) supplemented with *Mycobacterium tuberculosis* H37 Ra (Difco Laboratories, Detroit, MI, USA) on day 1 and 8. Control group (group C) was immunized with FCA alone. The animals were daily observed up to the end of the experiment. The day of injection was designed day 1. LXA4, from Cayman Chemical Company (Ann Arbor, MI, USA), was stored at -80°C until being diluted in phosphate buffered saline (PBS) immediately before use. Mice successfully induced with EAM were randomly assigned into three groups (each group n=10): non-treated group (group N), low-dose LXA4 group (10 μg/kg/day, group L) and high-dose LXA4 group (50 μg/kg/day, group H). LXA4 therapy started at the same time of immunization. The animals were administrated intraperitoneally for 3 weeks from day 1 to day 21 after immunization. Non-treated EAM animals received physiological saline.

Echocardiographic Studies

Echocardiography was conducted on day 21 post-myosin injection to assess the cardiac function and heart structure of the mice. Mice were anesthetized with an intraperitoneal injection of 40 mg/kg sodium pentobarbital (Beijing propbs Biotechnology Co., Ltd, Beijing, China). A 12 MHz probe was placed at the left 4th intercostal space for M-mode imaging using 2D echocardiography (Sono 550, Philips, Amsterdam, The Netherlands). Left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVEDs), and left ventricular posterior wall thickness (LVPW) were determined using the leading-edge method. Left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF) were calculated as previously described[16].

Histopathology

The body weight of mice was noted just before the surgical produce. After the echocardiographic analysis, the mice were sacrificed under sodium pentobarbital anesthesia, blood samples were obtained from abdominal aorta and the hearts were removed and weighed to calculate the ratio of HW/BW (mg/g). One portion heart was rinsed with phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde and embedded in paraffin (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China); the other portion was frozen at -80°C for protein studies. Embedded tissue was

cut on a microtome (3 μm thickness) and stained with Haematoxylin and Eosin (H&E) (Sigma-Aldrich, St. Louis, MO, USA), and evaluated by light microscopy.

Cytokine Measurement

Enzyme-linked immunosorbent assay (ELISA) was performed to detected serum levels of TNF-α and IL-6 as representative Th1 cytokines and IL-4, IL-10 as representative Th2 cytokine on day 21 with ELISA kits (BioSource International, Camarillo, CA, USA) according to manufacture's instructions.

Western Blot Analysis

Myocardial tissue samples were homogenized with lysis buffer. The protein concentrations were measured by the bicinchoninic acid assay. Proteins were denatured, separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane. After being blocked with 5% non-fat milk, blots were incubated with primary antibodies overnight at 4°C, and then exerted to incubation with the corresponding secondary antibodies at room temperature for 2 h. Membranes were developed with electrochemiluminescence (ECL) Western blot substrate (GE Healthcare, Buckinghamshire, UK) and Western blots were visualized on the Kodak Image Station (Carestream Health Inc., New York, NY, USA). Antibodies specific for p-IκBα, p-NF-κB p65, p-IKKα/β, IKKα, p-Akt, Akt, PI3K, p-PI3K and GAPDH were obtained from Cell signaling Technology (Danvers, MA, USA). Anti-IκBα and anti-NF-κB p65 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Statistical Analysis

Data analyses were performed using one-way analysis of variance (ANOVA) followed by the Newman-Keuls *t*-test to isolate significantly different values. Results were expressed as means ± standard deviation (SD). $p<0.05$ was considered statistically significant.

Results

Heart Weight/Body Weight Ratio

There was no death during the study period in all the groups. As manifested in Table I, HW and BW were recorded and HW/BW ratios were calculated to assess cardiac hypertrophy. After immunization, HW/BW ratio was significantly increased in-group N compared with group C ($p<0.01$). In contrast, there was a significant reduction in the HW/BW ratio in the high-dose LXA4-treated group compared with the non-treated group ($p<0.01$).

LXA4 Treatment Improved the Cardiac Function

The heart function and cardiac structure in all groups were evaluated by echocardiographic analysis on day 21 after immunization. As demonstrated in Figure 1, LVEDs and LVEDd were significantly enhanced ($p<0.05$), while LVFS was markedly impaired ($p<0.01$) in-group N compared with group C. However, LXA4 intervention decreased LVEDs and LVEDd and increased LVFS compared with group N. Also, LVEF was significantly decreased ($p<0.01$) in the group N compared with the group C, while treatment with LXA4 inhibited the decrease. These results indicated that the left ventricular dysfunction and severely altered heart structure were observed following EAM, and LXA4 treatment effectively suppressed the progression of left ventricular remodeling.

Effect of LXA4 on Myocarditis-Affected Areas

Histopathologic analysis was performed on heart removed from experimental mice on 21 days post-immunization. As illustrated in Figure 2, the typical signs of myocarditis with severe in-

Table I. HW, BW and HW/BW ratio in different groups.

	HW (mg)	BW (g)	HW/BW (mg/g)
Group C	131.1 ± 3.9	39.2 ± 1.7	3.34 ± 0.32
Group N	179.8 ± 9.8**	28.4 ± 1.8**	6.33 ± 0.71**
Group L	165.4 ± 1.7	29.2 ± 1.2	5.66 ± 0.43
Group H	149.9 ± 6.8##	32.1 ± 1.4##	4.70 ± 0.38##

Values are expressed as means ± SD, n = 10; **$p<0.01$ vs. group C, #$p<0.05$, ##$p<0.01$ vs. group N.

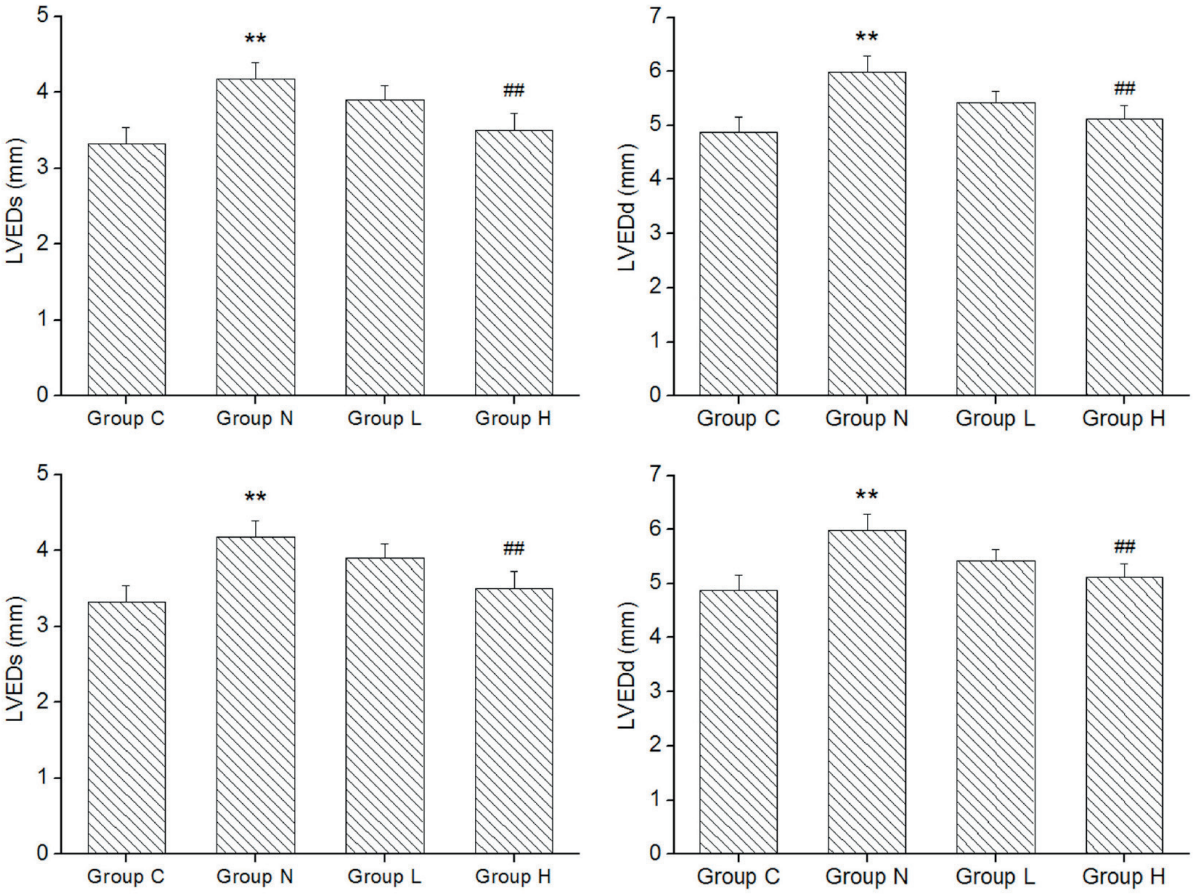

Figure 1. Echocardiography examination of experimental mice. Values are expressed as means ± SD, n=10; $^{*}p<0.05$, $^{**}p<0.01$ *vs.* group C, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* group N. LVEDd: left ventricular end-diastolic diameter; LVEDs: left ventricular end-systolic diameter; LVFS: left ventricular fractional shortening; LVEF: left ventricular ejection fraction.

flammatory lesions showed myocardial necrosis, degeneration and infiltration by inflammatory cells, which were observed in-group N. However, LXA4 treatment significantly attenuated the severity of the disease as evaluated by detecting the typical signs of myocarditis.

LXA4 Treatment Regulated Th1/Th2 Cytokine Balance

ELISA was performed to investigate the changes of Th1 and Th2 cytokine balance in different groups. As shown in Figure 3, the production of Th1-type cytokines TNF-α and IL-6 were significantly elevated, accompanied by a decrease in the production of Th2 cytokines IL-4 and IL-10 in group N compared with group C ($p<0.01$). However, LXA4 intervention significantly reversed those changes. The therapeutic effect of LXA4 on EAM might be due to its suppression of Th1 immune response and augmentation of Th2 response, as confirmed by a regulation of Th1/Th2 cytokine production.

LXA4 Treatment Suppressed NF-κB Activation

The NF-κB is reported to play an important role in regulating the induction of a variety of pro-inflammatory cytokines in cardiac inflammation. Therefore, we investigated whether LXA4 affected NF-κB signaling in EAM. As manifested in Figure 4, EAM indeed enhanced the phosphorylation of IκBα and NF-κB p65 as well as led to the degradation of IκBα and NF-κB p65 in the group N. However, LXA4 treatment effectively suppressed phosphorylation of both IκBα and NF-κB p65. We also evaluated the phosphorylation status of IKKα/β proteins, which are upstream of the IκB/NF-κB complex. As illustra-

ted in Figure 5, IKKα/β protein phosphorylation was dramatically increased in the group N and this phosphorylation was strongly inhibited by LXA4 treatment. These results indicated that NF-κB signaling pathway is affected by LXA4, which makes it a useful anti-inflammation therapy.

LXA4 Treatment Inhibited PI3K/Akt Activation

The PI3K/Akt pathway is reported to be important for NF-κB activation via modulation of IκBα phosphorylation and degradation[17,18]. To investigate whether LXA4-mediated inhibition of NF-κB activation involved the PI3K/Akt pathway, we examined the effect of LXA4 on PI3K/Akt activation in EAM. As demonstrated in Figure 6, the phosphorylation of PI3K and Akt in the myocardial tissue elevated significantly in the group N compared with the group C, while LXA4 treatment reversed these events.

Discussion

Acute myocarditis is a potentially lethal disease, and frequently precedes the development of dilated cardiomyopathy[19,20]. Although significant progress has been achieved recently, there is still much to be done to improve the therapy for this disease. In this study, we used a murine model of EAM, which mimics human myocarditis complications, as well as a variety of biomarker to monitor disease evolution. The results demonstrated that treatment with LXA4

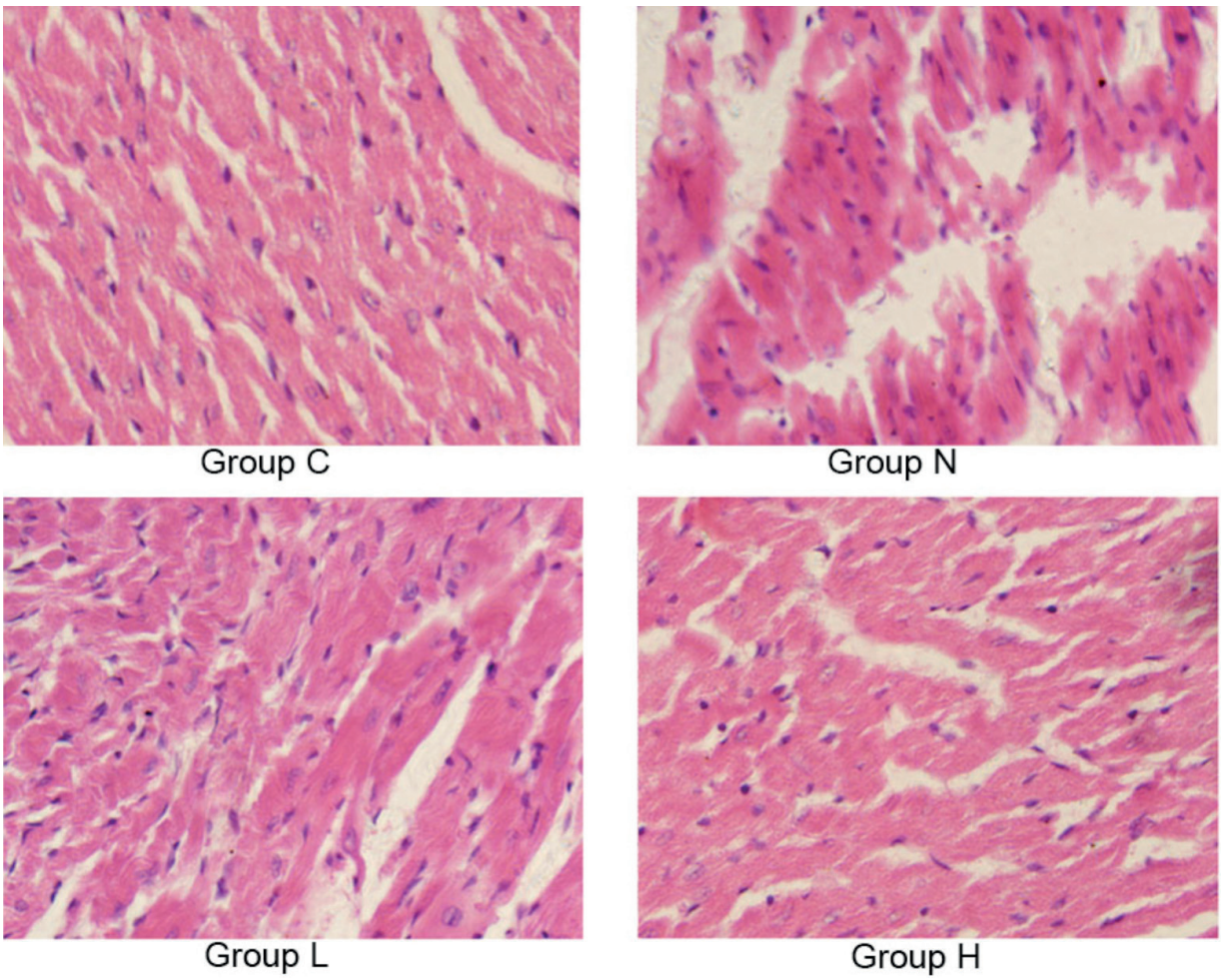

Figure 2. Histological analysis of the myocardium in different groups by H&E staining (×100). Group C: control group was immunized with FCA alone. Group N: black group was non-treated. Group L: test group was low-dose LXA4 group (10 μg/kg/day). Group H: test group was high-dose LXA4 group (50 μg/kg/day).

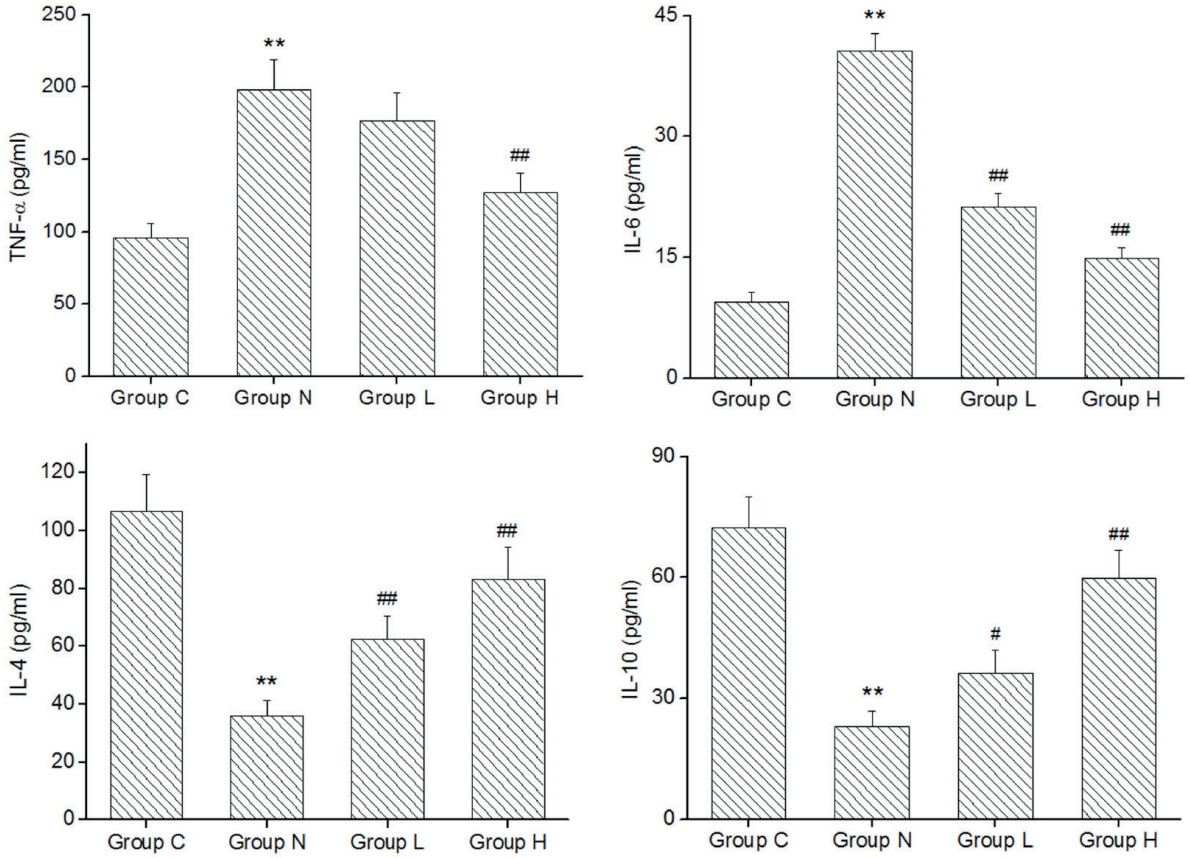

Figure 3. Levels of cytokine of Th1 (TNF-α and IL-6) and Th2 (IL-4 and IL-10) evaluated by ELISA. Values are expressed as means ± SD, n = 10; $^{*}p<0.05$, $^{**}p<0.01$ *vs.* group C, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* group N.

significantly attenuated the severity of EAM by regulating inflammatory response, NF-κB and PI3K/Akt signaling pathway. Myocarditis is associated with inflammation and cytokine modulation, and myocarditis was the state of some derangements of the immune system, such as T-cell dysfunction and the excessive production of detrimental cytokine[16,21]. Overproduction of pro-inflammatory cytokine plays a pivotal role in the pathogenesis of myocarditis[22]. Also, myocarditis was a $CD4^{+}$ T-cell-mediated disease, and the imbalance between Th1 and Th2 is believed to be responsible for the initiation and mediation of myocarditis[23]. LXA4 is known for its powerful anti-inflammatory function. LXA4 can suppress antigen-presenting cell functions and regulate cytokine-driven immune reactions towards Th2 responses[24,25]. LXA4 treatment attenuates ischemia/reperfusion injury by modulating Th1/Th2 balance accompanied by up-regulating the level of the anti-inflammatory cytokines IL-4 and IL-10 and downregulating the level of IL-2 and TNF-α[26]. Also, LXA4 alleviates acute rejection with a parallel shift from Th1 to Th2 responses in solid organ transplantation[25]. In this study, the production of Th1-type cytokines (TNF-α and IL-6) was significantly increased, accompanied by a decrease in the production of Th2 cytokines (IL-4 and IL-10) in EAM rats. LXA4 treatment significantly down-regulated the increased serum levels of Th1 cytokine and up-regulated Th2 cytokine significantly in myocarditis mice. It is conceivable that LXA4 has immunosuppressive properties that result in a reduction of inflammation associated with the shift of Th1 cells towards Th2 cells, and ultimately in a reduction in autoimmune-mediated myocardial injury. LXA4 finally suppressed the releasing of inflammatory cytokines associated with the shift of Th1 to Th2 cytokine balance. NF-κB signaling regulation is considered as an important therapeutic target for inflammatory

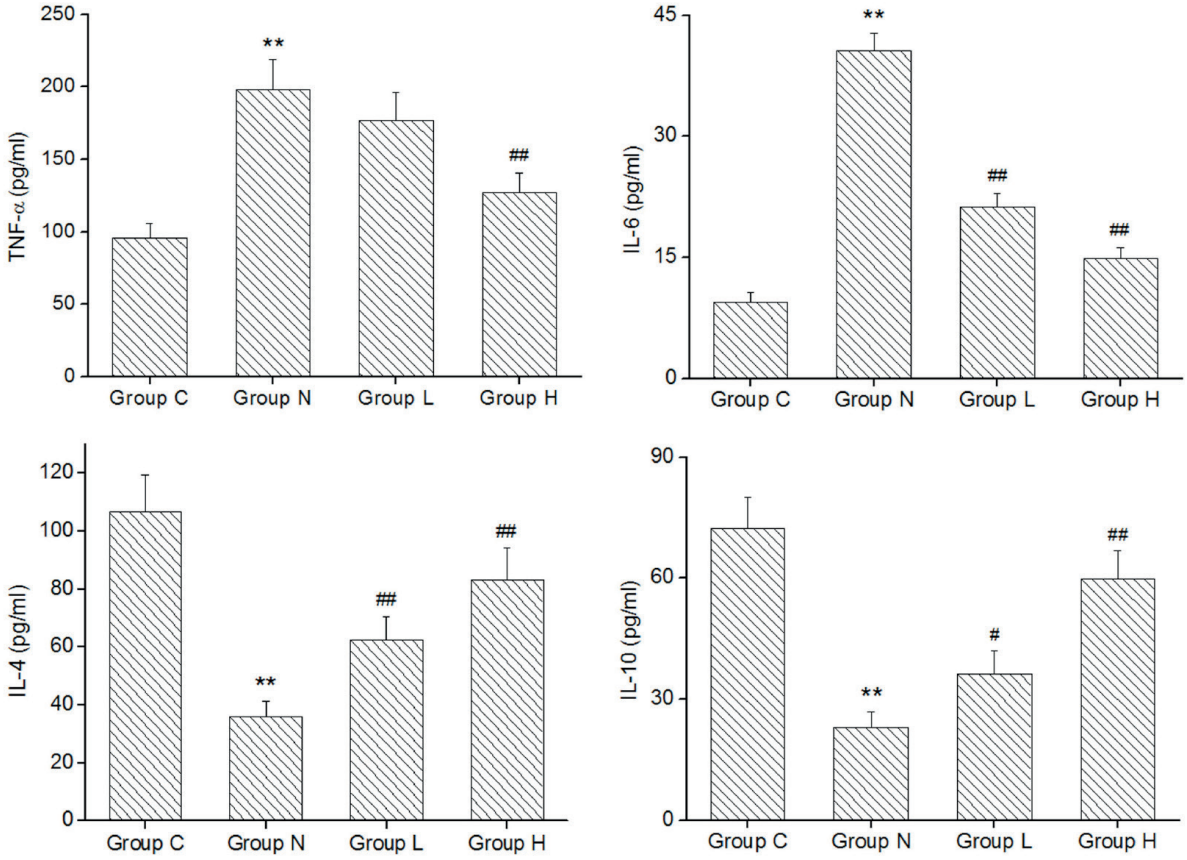

Figure 4. Effects of LXA4 on NF-κB activation. ***(A)*** Representative Western blot for p-NF-κB p65, NF-κB p65, p-IκBα and IκBα. ***(B)*** Relative level of p-NF-κB p65 protein (fold change to total NF-κB p65 protein level). ***(C)*** Relative level of p-IκBα protein (fold change to total IκBα protein level). Values are expressed as means ± SD, n = 3; $^{*}p<0.05$, $^{**}p<0.01$ *vs.* group C, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* group N.

diseases because inappropriately activated NF-κB signaling contributes to inflammatory disorders[27]. In unstimulated cells, NF-κB complex is localized and inactivated in the cytoplasm by binding to the inhibitory protein IκB[28]. IκB kinase (IKK) can phosphorylate IκB and lead to its degradation. NF-κB is then released to translocate to nucleus and activate transcription[29]. NF-κB activity has been proved to be essential for the production of pro-inflammatory cytokines in cardiac myocytes[30]. There is direct evidence that inhibiting NF-κB activity by NF-κB inhibitor can block the production of pro-inflammatory cytokine in cardiac tissues or myocytes, thus preventing the development of myocarditis[31,32]. In this study, we investigated the effect of LXA4 on the activity of NF-κB in EAM mice. Results demonstrated that LXA4 treatment reduced degradation and phosphorylation of IκBα and NF-κB p65, prevented nuclear translocation of NF-κB p65, and suppressed phosphorylation of the upstream signaling protein IKKα/β, indicating that LXA4 affected the IKK complex in the NF-κB signaling pathway. The PI3K/Akt pathway is implicated in the regulation of a series of physiological activities and can be activated by a variety of extracellular stimuli[33]. It is reported that PI3K/Akt pathway may be an important therapeutic target for many inflammatory diseases, including diovascular diseases[30,34]. The pathway in comprised of two main driving molecules: PI3K3Kinase (PI3K) and AKT. PI3K is a member of a subfamily of lipid kinases that phosphorylate the 3-hydroxyl group of phosphoionositides and involved in many physiological processes[35,36]. AKT, the downstream target of PI3K, is cytosolic protein whose translocation is induced by the binding to PI3K products and

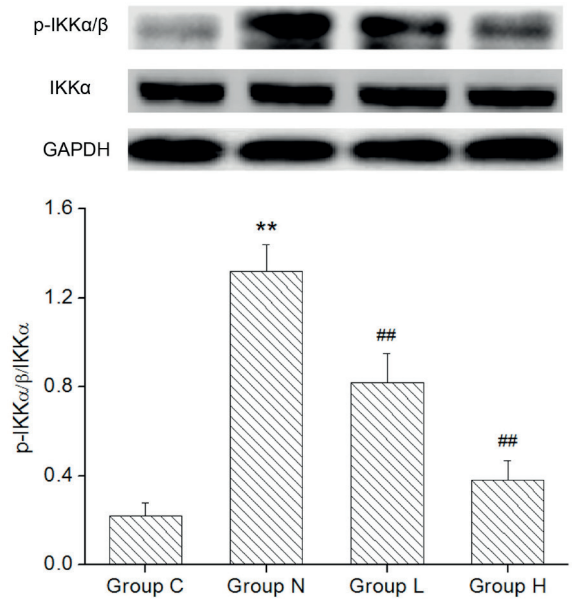

Figure 5. Effects of LXA4 on the phosphorylation status of IKKα/β protein. Values are expressed as means ± SD, n=3; $^{*}p<0.05$, $^{**}p<0.01$ *vs.* group C, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* group N.

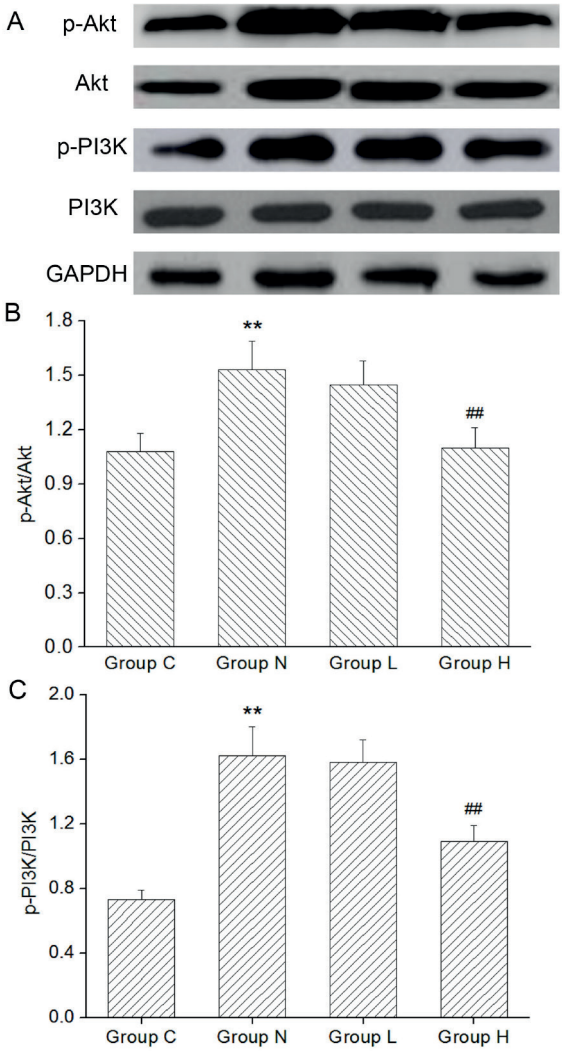

Figure 6. Effects of LXA4 on PI3K/Akt activation. ***(A)*** Representative Western blot for p-Akt, Akt, p-PI3K and PI3K. ***(B)*** Relative level of p-Akt protein (fold change to total Akt protein level). ***(C)*** Relative level of p-PI3K protein (fold change to total PI3K protein level). Values are expressed as means ± SD, n=3; $^{*}p<0.05$, $^{**}p<0.01$ *vs.* group C, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* group N.

acts as a key protein mediator for a wide range of cellular processes[37,38]. The PI3K/Akt pathway is reported to be important for NF-κB activation; it can influence NF-κB activity by promoting IKK activation and subsequent phosphorylation and degradation of IκBα, which activates the RelA/p65 subunit of NF-κB in some systems[30,39,40]. In this study, Western blot analysis demonstrated that the phosphorylation of PI3K and Akt in the myocardial tissue significantly increased in the non-treated group, LXA4 treatment dramatically repressed these events. These results indicated that LXA4 alleviated EAM at least partly via modulating PI3K/Akt pathway.

Conclusions

The LXA4 administration greatly reduces the severity of EAM and the mechanism can be at least partly explained by removing of pro-inflammatory cytokines, regulating the transition of helper T-cell balance from Th1 to Th2, and inhibiting PI3/Akt and NF-κB signaling pathway. Therefore, LXA4 may be a promising agent for the clinical treatment of myocarditis and it represents a paradigm of immunosuppressive therapy on the immune-mediated disease.

Ethical Approval

The research was conducted in accordance with the Declaration of Helsinki and the United National Institutes of Health.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Caforio AL, Goldman JH, Haven AJ, Baig KM, McKenna WJ. Evidence for autoimmunity to myosin and

other heart-specific autoantigens in patients with dilated cardiomyopathy and their relatives. Int J Cardiol 1996; 54: 157-163.

2) Brown CA, O' Connell JB. Myocarditis and idiopathic dilated cardiomyopathy. Am J Med 1995; 99: 309-314.

3) Cihakova D, Rose NR. Pathogenesis of myocarditis and dilated cardiomyopathy. Adv Immunol 2008; 99: 95-114.

4) Gonnella PA, Del Nido PJ, McGowan FX. Oral tolerization with cardiac myosin peptide (614-629) ameliorates experimental autoimmune myocarditis: role of STAT 6 genes in BALB/CJ mice. J Clin Immunol 2009; 29: 434-443.

5) Kishimoto C, Nimata M, Okabe TA, Shioji K. Immunoglobulin treatment ameliorates myocardial injury in experimental autoimmune myocarditis associated with suppression of reactive oxygen species. Int J Cardiol 2013; 167: 140-145.

6) Sukumaran V, Watanabe K, Veeraveedu PT, Ma M, Gurusamy N, Rajavel V, Suzuki K, Yamaguchi K, Kodama M, Aizawa Y. Telmisartan ameliorates experimental autoimmune myocarditis associated with inhibition of inflammation and oxidative stress. Eur J Pharmacol 2011; 652: 126-135.

7) Martín R, Cordova C, San Román JA, Gutierrez B, Cachofeiro V, Nieto ML. Oleanolic acid modulates the immune-inflammatory response in mice with experimental autoimmune myocarditis and protects from cardiac injury. Therapeutic implications for the human disease. J Mol Cell Cardiol 2014; 72: 250-262.

8) Romano M. Lipid mediators: lipoxin and aspirin-triggered 15-epi-lipoxins. Inflamm Allergy Drug Targets 2006; 5: 81-90.

9) Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxin biosynthesis: an update and role in anti-inflammation and pro-resolution. Prostaglandins Other Lipid Mediat 2002; 68-69: 433-455.

10) Poeckel D, Zemski Berry KA, Murphy RC, Funk CD. Dual 12/15- and 5-lipoxygenase deficiency in macrophages alters arachidonic acid metabolism and attenuates peritonitis and atherosclerosis in ApoE knock-out mice. J Biol Chem 2009; 284: 21077-21089.

11) Huang H, Song TJ, Li X, Hu L, He Q, Liu M, Lane MD, Tang QQ. BMP signaling pathway is required for commitment of C3HOT1/2 pluripotent stem cells to the adipocyte lineage. Proc Natl Acad Sci USA 2009; 106: 12670-12675.

12) Baker N, O'Meara SJ, Scannell M, Maderna P, Godson C. Lipoxin A4: anti-inflammatory and anti-angiogenic impact on endothelial cells. J Immunol 2009; 182: 3819-3826.

13) Levy BD. Lipoxins and lipoxin analogs in asthma. Prostaglandins Leukot Essent Fatty Acids 2005; 73: 231-237.

14) Sun T, Yu E, Yu L, Luo J, Li H, Fu Z. LipoxinA(4) induced antinociception and decreased expression of NF-kappaB and pro-inflammatory cytokines after chronic dorsal root ganglia compression in rats. Eur J Pain 2012; 16: 18-27.

15) Fiore S, Antico G, Aloman M, Sodin-Semrl S. Lipoxin A4 biology in the human synovium. Role of the ALX signaling pathways in modulation of inflammatory arthritis. Prostaglandins Leukot Essent Fatty Acids 2005; 73: 189-196.

16) Liu X, Zhu X, Wang A, Fan H, Yuan H. Effects of angiotensin-II receptor blockers on experimental autoimmune myocarditis. Int J Cardiol 2009; 137: 282-288.

17) Venkatachalam K, Mummidi S, Cortez DM, Prabhu SD, Valente AJ, Chandrasekar B. Resveratrol inhibits high glucose-induced PI3K/Akt/ERK-dependent interleukin-17 expression in primary mouse cardiac fibroblasts. Am J Physiol Heart Circ Physiol 2008; 294: H2078-H2087.

18) Li XQ, Cao W, Li T, Zeng AG, Hao LL, Zhang XN, Mei QB. Amlodipine inhibits TNF-alpha production and attenuates cardiac dysfunction induced by lipopolysaccharide involving PI3K/Akt pathway. Int Immuno Pharmacol 2009; 9: 1032-1041.

19) Li Y, Heuser JS, Kosanke SD, Hemric M, Cunningham MW. Cryptic epitope identified in rat and human cardiac myosin S2 region induces myocarditis in the Lewis rat. J Immunol 2004; 172: 3225-3234.

20) Liu XM, Li B, Wang WK, Zhang C, Zhang MX, Zhang Y, Xia YF, Dong Z, Guo Y, An FS. Effects of HMG-CoA reductase inhibitor on experimental autoimmune myocarditis. Cardiovasc Drugs Ther 2012; 26: 121-130.

21) Mehra VC, Ramgolam VS, Bender JR. Cytokines and cardiovascular disease. J Leukoc Biol 2005; 78: 805-818.

22) Shioi T, Matsumori A, Sasayama S. Persistent expression of cytokines in the chronic stage of viral myocarditis in mice. Circulation 1996; 94: 2930-2937.

23) Yuan ZY, Liu Y, Liu Y, Zhang JJ, Kishimoto C, Wang YN, Ma AQ, Liu ZQ. PPAR-gamma ligands inhibit the expression of inflammatory cytokines and attenuate autoimmune myocarditis. Chin Med J (Engl) 2004; 117: 1253-1255.

24) Parkinson JF. Lipoxin and synthetic lipoxin analogs: an overview of anti-inflammatory functions and new concepts in immunomodulation. Inflamm Allergy Drug Targets 2006; 5: 91-106.

25) Liao W, Zeng F, Kang K, Qi Y, Yao L, Yang H, Ling L, Wu N, Wu D. Lipoxin A4 attenuates acute rejection via shifting TH1/TH2 cytokine balance in rat liver transplantation. Transplant Proc 2013; 45: 2451-2454.

26) Zhou XL, Yang QS, Ni SZ, Tu XP, Zhao Y, Xu B, Pan ZQ, Shen J. Protective effects of lipoxin A4 in testis injury following testicular torsion and detorsion in rats. Mediat Inflamm 2014; 2014: 898056.

27) Jeon YJ, Kim BH, Kim S, Oh I, Lee S, Shin J, Kim TY. Rhododendrin ameliorates skin inflammation through inhibition of NF-κB, MAPK, and PI3K/Akt signaling. Eur J Pharmacol 2013; 714: 7-14.

28) Hayden, MS, Ghosh S. NF-kappaB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 2012; 26: 203-234.

29) Liu Z, Zhang HM, Yuan J, Lim T, Sall A, Taylor GA, Yang D. Focal adhesion kinase mediates the in-

terferon-gamma-inducible GTPase-induced phosphatidylinositol 3-kinase/Akt survival pathway and further initiates a positive feedback loop of NF-kappaB activation. Cell Microbiol 2008; 10: 1787-1800.

30) Song YX, Ge W, Cai HB, Zhang HC. Curcumin protects mice from coxsackievirus B3-induced myocarditis by inhibiting the phosphatidylinositol 3 kinase/Akt/nuclear factor-κB pathway. J Cardiovasc Pharmacol Ther 2013; 18: 560-569.

31) Matsumori A, Nunokawa Y, Yamaki A, Yamamoto K, Hwang MW, Miyamoto T, Hara M, Nishio R, Kitaura-Inenaga K, Ono K. Suppression of cytokines and nitric oxide production, and protection against lethal endotoxemia and viral myocarditis by a new NF-kappaB inhibitor. Eur J Heart Fail 2004; 6: 137-144.

32) Gui J, Yue Y, Chen R, Xu W, Xiong S. A20 (TNFAIP3) alleviates CVB3-induced myocarditis via inhibiting NF-kB signaling. PLoS One 2012; 7: e46515.

33) Garat CV, Crossno JT, Jr, Sullivan TM, Reusch JE, Klemm DJ. Inhibition of phosphatidylinositol 3-kinase/Akt signaling attenuates hypoxia-induced pulmonary artery remodeling and suppresses CREB depletion in arterial smooth muscle cells. J Cardiovasc Pharmacol 2013; 62: 539-548.

34) Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, Lawitts J, Cantley LC, Izumo S. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. EMBO J 2000; 19: 2537-2548.

35) Dobbin ZC, Landen CN. The importance of the PI3K/AKT/MTOR pathway in the progression of ovarian cancer. Int J Mol Sci 2013; 14: 8213-8227.

36) Martini M, Ciraolo E, Gulluni F, Hirsch E. Targeting PI3K in cancer: any good news? Front Oncol 2013; 3: 108.

37) Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307: 1098-1101.

38) Franke TF, Cantley LC. Apoptosis. A bad kinase makes good. Nature 1997; 390: 116-117.

39) Feng D, Wang W, Dong Y, Wu L, Huang J, Ma Y, Zhang Z, Wu S, Gao G, Qin H. Ceftriaxone alleviates early brain injury after subarachnoid hemorrhage by increasing excitatory amino acid transporter 2 expression via the PI3K/Akt/NF-κB signaling pathway. Neuroscience 2014; 268: 21-32.

40) Xia M, Tong JH, Ji NN, Duan ML, Tan YH, Xu JG. Tramadol regulates proliferation, migration and invasion via PTEN/PI3K/AKT signaling in lung adenocarcinoma cells. Eur Rev Med Pharmacol Sci 2016; 20: 2573-2580.