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Original Research Article

Protective effect of maternal exposure to α-lipoic acid during pregnancy and lactation on susceptibility to OVA-induced neonatal asthma

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Abstract

Purpose: *To investigate the beneficial effect of alpha-lipoic acid (ALA) during pregnancy and lactation on susceptibility to ovalbumin (OVA)-induced neonatal asthma, and the mechanism of involved.*
Methods: *Pregnant BALB/c mice were administered ALA (1 % mixed with mouse chow) or standard mouse chow from 6th day of gestation to 21st day of lactation (postnatal). The offspring (neonatal pups) from the OVA and ALA+OVA groups were sensitized on 1st, 7th and 14th postnatal days (PNDs) via intraperitoneal (i.p.) injection of OVA (0.5 µg). Control mice pups were not exposed to OVA. On PND 21, all pubs were again exposed to 1 % OVA aerosol using a nebulizer.*
Results: *Neonatal mice exposed to ALA showed a significant decline ($p < 0.05$) in the number of inflammatory cells (eosinophils), levels of inflammatory markers (IL-4, IL-13, IL-5 and TNF-α) as well as OVA-specific IgE and total IgE, when compared to neonatal mice from pregnant mice that did not receive ALA (control). Moreover, the antioxidant profiles of ALA-treated mice offspring were significantly improved ($p < 0.05$). Marked downregulation ($p < 0.05$) of the protein expressions of NF-κB p-p65 subunit and TNF-α were observed in ALA-treated mice pups.*
Conclusion: *ALA exposure during pregnancy (maternal exposure) markedly decreases OVA-induced asthmatic airway inflammatory response in pups. Thus, ALA might be beneficial for use along with standard anti-asthmatic drugs in the management of pediatric asthmatic patients*

Keywords: *α-Lipoic acid, Asthma, Ovalbumin, Inflammation, Neonatal*

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INTRODUCTION

Asthma is a chronic respiratory inflammatory disease that results in elevated airway inflammation, epithelial cell damage and thickening of the airway wall, and bronchial hyper-responsiveness (airway remodeling), followed by narrowing or closure of the windpipe (airway obstruction). It is one of the predominant respiratory diseases that affect more than 300 million people globally, with the numbers increasing every year. It has been predicted that approximately 400 million people will be affected by asthma by the year 2025 [1, 2]. The major

causative agents of asthma are allergens (dust, pollen and fur or air pollutants); chemicals (OVA and LPS); viruses, oxidants/exercise, emotional disturbance, food additives, endocrinal factors and hyperventilation [3]. Many studies have indicated that oxidative stress (imbalance between free radicals and antioxidants), inflammation, as well as reduced immunity are implicated in asthma. However, not much is known about the in-depth pathophysiology of asthma [4, 5]. The current treatment regimens involve mostly inhalation drugs such as corticosteroids, anti-IgE drugs (monoclonal IgE antibodies), β2-adrenergic agonist, antileukotrienes/cholinergic (individual or combinational therapy), in addition to bronchial thermoplasty procedure. However, long term usage of these drugs might result in severe adverse effects such as hormonal imbalance, renal and hepatic damage, as well as sub-optimal adherence (insensitivity) to asthma [6 - 8]. Thus, there is need for an alternative natural anti-asthmatic agent with potent antioxidative, anti-inflammatory and immuno-modulatory (anti-allergic) properties, and minimum or no side effects.

Alpha-lipoic acid (ALA) or dihydro lipoic acid (reduced form of ALA) is an amphipathic antioxidant molecule synthesized mainly in mitochondria of liver, kidney, and heart cells. It acts as a cofactor for many enzymes. It possesses very good metal ion-chelating property due to the presence of two sulfhydryl groups, and it exhibits free radical-scavenging/quenching activity owing to the presence of free hydroxyl groups in its structure [9,10]. Alpha-lipoic acid (ALA) has attracted a lot of research interest owing to its potent antioxidant activity and its anti-lipid peroxidation, anti-inflammatory, immunomodulatory, anti-allergic, antidiabetic, antitumor, wound-healing, cardioprotective, renoprotective, neuroprotective and hepatoprotective properties [10 - 12]. Previously, Cho *et al* [13] and Park *et al* [14] reported that lipoic acid treatment significantly lowered allergic inflammatory response and airway remodeling in a mouse asthma model [13,14]. However, the study did not investigate the effect of ALA on maternal exposure to ALA during pregnancy and lactation, with respect to OVA-induced asthma in the mouse model. Therefore, the current animal study was aimed at investigating the beneficial effect of ALA during pregnancy and lactation as regards susceptibility to neonatal asthma induced with OVA in a mouse model, as well as the involvement of inflammatory NF-κB signaling pathway in the process.

EXPERIMENTAL

Chemicals and reagents

Ovalbumin, aluminum hydroxide, Tris-HCl buffered solution and Wright-Giemsa stain were purchased from Sigma-Aldrich (MA, USA). Physiological saline, ketamine, and xylazine were bought from Biosino Biotechnology and Science Inc. Ltd (Beijing, China). All primary and secondary antibodies used in this study were products of Abcam (Cambridge, UK).

Animals

A total of 6 healthy pregnant BALB/c mice from the same litter (mean weight = 28 ± 32 g) were purchased from a local laboratory experimental animal centre in Wuhan, China. All mice were housed individually in a polycarbonate cage at optimal laboratory condition (12-h light/12-h dark cycle at 55 - 60 % humidity and temperature of 22 - 24 °C. Standard mouse chow and drinking water were made available *ad libitum*. The animal experiment was approved by the Ethical Committee of Taihe Hospital, Hubei University of Medicine (approval no. HU-14/2014-03), and the animals were handled in line with the guidelines stipulated by NIH (MA, USA) [10].

Animal grouping

The pregnant mice were administered ALA (1 % mixed with mouse chow; n = 2) or standard mouse chow only (n = 4) from gestation day 6 to lactation day 21 (postnatal). After parturition, 18 healthy offspring (neonatal pups) were chosen for further studies. The 18 neonatal mice comprised 6 neonatal mice from mother mice administered ALA + standard mouse chow, and 12 neonatal neonatal mice from mother mice fed only with standard mouse chow (without ALA). These 18 neonatal mice were divided into 3 groups: control group (sensitized with saline) without any OVA exposure, positive control (OVA-treated group) and ALA + OVA group. The OVA-treated groups were sensitized on the 1st, 7th and 14th postnatal days (PNDs) via intraperitoneal (i.p.) injection of 0.5 µg OVA and 200 µg aluminum hydroxide (in saline). On PND 21, neonatal pups in the OVA group and ALA + OVA group were again challenged with 1 % OVA aerosol for 30 min using a nebulize, as indicated by Dong *et al* [15].

Collection of samples

On the 22nd day, following an overnight fast, the neonatal mice were anesthetized by i.p. injection of ketamine (60 mg/kg) and xylazine (20 mg/kg),

and sacrificed via decapitation. Then, bronchoalveolar lavage fluid (BALF) was collected from the left lungs [15]. For this purpose, the chest cavity was opened and a cannula was inserted into the trachea of the left lungs and lavaged with 0.5 mL of saline. The fluid (BALF) was gently aspired and centrifuged at 8000 rpm for 5 min at 4°C, and the resultant supernatant was used for various biochemical analyses. The lung tissue was excised immediately and weighed using a standard animal weighing machine, and portions of the tissue was thereafter dried at 90 °C for 72 h, after which the ratio of wet weight/dry weight was calculated [16]. Then, the remaining fresh lung tissues were chopped into small bits and homogenized in Tris-Hcl buffered solution (Ph 7.4). The homogenate was centrifuged at 12,000 rpm for 8 min at 4°C, and the resultant supernatant was used for further analysis.

Inflammatory cell count

The BALF was used to determine the various inflammatory cell counts, including eosinophils and total cells, by staining with Wright-Giemsa and counting using cytospin techniques [17].

Determination of inflammatory cytokines

The concentration of various inflammatory markers i.e interleukins (IL)-5, IL-4, IL-13) and tumor necrosis factor-alpha (TNF-α) in BALF were determined using ELISA commercial kits (R and D Systems, MN, USA), based on manufacturers protocol.

Assay of immunoglobulins (Ig)

Serum total immunoglobulin E (IgE) and OVA-specific IgE were measured using mouse-specific commercial ELISA kit (MyBioSource, CA, USA), in line with the manufacturer's procedure.

Assay of antioxidant enzyme activities and lipid-peroxidation products

The activities of catalase (CAT), superoxide dismutase (SOD), as well as levels of lipid peroxidation product malondialdehyde (MDA) were determined in lung tissue homogenate using Bio Assay kit (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., Beijing, China).

Determination of protein expressions

Nuclear and cytoplasmic fractions of lung homogenate were isolated using Cell Fractionation kit (Abcam; Cambridge, UK). The protein in both isolated fractions (cytoplasmic and nuclear) were extracted using lytic buffer solution, and the total protein levels were estimated with BCA protein assay kit (Abcam, Cambridge, UK). Equal concentrations of protein samples (30-µg) from each fraction (nuclear and cytoplasmic) were resolved using 8 % SDS-polyacrylamide gel electrophoresis. Then, the proteins were transferred onto PVDF membranes, and the membranes were blocked by incubation with blocking solution containing Tween 20, Tris-HCl, NaCl and skimmed milk. Thereafter the membranes were probed with primary antibodies i.e., rabbit monoclonal anti-TNF-α (1:1000 dilution), anti-NF-κB p-p65 (1:800) and β actin/histone H3 (1:1500; housekeeping) for 8 h at 4°C. Then, the membranes were incubated with secondary antibody (HRP-conjugated anti-goat IgG monoclonal antibody) at 1:10000 dilution for 2 h at 4°C. The protein-resolved PVDF membranes were developed using an ECL kit, and the protein bands were captured using a digital camera attached to the Chem Doc imaging Detection system (GE Health life Science; IL, USA) with Image Pro-plus imaging software (Media Cybernetics; MD, USA).

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Differences amongst groups were determined using one-way ANOVA, followed by Duncan's multi-comparison test. All statistical analyses were carried out with SPSS software version 21 (IBM Inc, NY, USA).

RESULTS

Effect of ALA on lung oedema (water content)

The ratio of wet weight/dry weight was calculated as an index of pulmonary oedema. Neonatal mice exposed to OVA showed markedly higher lung wet-to-dry weight ratio (6.25 ± 0.09) than control mice (4 ± 0.06). However, neonatal mice from ALA-treated mother mice had decreased lung wet-to-dry weight ratio (4.75 ± 0.07), when compared to OVA-induced mice.

Effect of ALA on inflammatory cell count

The effect of ALA on the severity of airway inflammation was measured by determining the mean inflammatory cell counts in BALF of neonatal mice exposed to OVA. As shown in Figure 1, the levels of total inflammatory cell count and eosinophils were significantly

increased ($p < 0.05$) in OVA-sensitized asthmatic mice. However, neonatal pups treated with ALA had significant reductions ($p < 0.05$) in the number of total inflammatory cells and eosinophils, when compared to OVA-exposed mice.

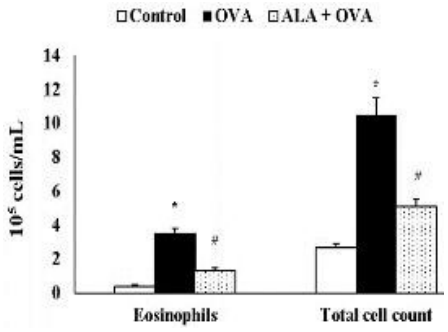

Figure 1: Impact of ALA on various inflammatory cell counts in BALF of neonatal mice exposed to OVA. Values are presented as mean ± SEM. $^{*}P < 0.05$, compared with control; $^{\#}p < 0.05$, compared with OVA

Effect of ALA on levels of inflammatory markers

The effect of ALA on various inflammatory markers in BALF of neonatal mice exposed to OVA is presented in Table 1. There were significant increases ($p < 0.05$) in the mean concentrations of various inflammatory markers (IL-4, IL-5, IL-13, and TNF-α) in OVA-treated asthmatic neonatal mice, relative to control neonatal mice. In contrast, the concentrations of these inflammatory markers (IL-4, IL-5, IL-13, and TNF-α) were significantly reduced ($p < 0.05$) to near normal levels in neonatal mice from ALA-exposed mother mice.

Effect of ALA on levels of immunoglobulins E

As indicated in Figure 2, the levels of total immunoglobulin E and OVA-specific immunoglobulin E (IgE) were markedly increased in neonatal mice from OVA-exposed mother mice ($p < 0.05$). However, the levels of total immunoglobulin E (IgE) and OVA-specific IgE, were decreased upon treatment with ALA ($p < 0.05$).

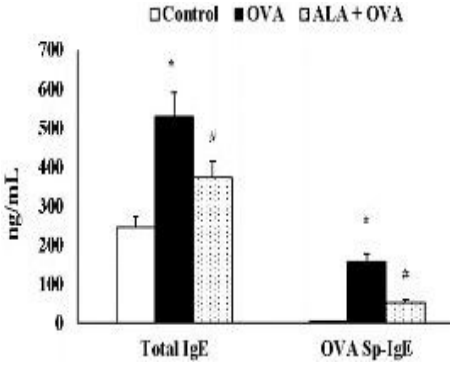

Figure 2: Effect of ALA on the levels of total immunoglobulin E (IgE) and OVA-specific immunoglobulin E (OVA-Sp-IgE) in BALF of neonatal mice exposed to OVA. Values are shown as mean ± SEM. $^{*}P < 0.05$, compared with control; $^{\#}p < 0.05$, compared with OVA

Effect of ALA on levels of antioxidants and MDA

The effect of ALA on antioxidant status (antioxidant enzymes) and lipid peroxidation products (MDA) in lung tissue homogenate of neonatal mice exposed to OVA is presented in Table 2. The activities of CAT and SOD were significantly decreased in OVA-sensitized mice (born and fed by mother mice exposed to OVA), while MDA levels were elevated ($p < 0.05$). However, these neonatal mice born and fed by mother mice treated with ALA had very marked increases in the activities of antioxidant enzymes (CAT and SOD), and reductions in MDA levels, when compared with those of OVA-induced neonatal mice ($p < 0.05$).

Table 1: Effect of ALA on inflammatory markers in control and OVA-exposed neonatal mice

Group	IL-4 (pg/ml)	IL-5 (pg/ml)	IL-13 (pg/ml)	TNF-α (pg/ml)
Control	122 ± 13.0	155 ± 16.5	162 ± 18.0	88.5 ± 10.0
OVA	565 ± 60.5*	610 ± 55.4*	632 ± 71.5*	695.0 ± 73.0
ALA + OVA	240 ± 12.5$^{\#}$	255 ± 15.8$^{\#}$	227 ± 24.3$^{\#}$	310.5 ± 32.0

Data are shown as mean ± SEM. $^{*}P < 0.05$, compared with control; $^{\#}p < 0.05$, compared with OVA

Table 2: Effect of ALA on antioxidant status and lipid peroxidation in control and OVA-sensitized neonatal mice

Group	CAT (U/mg)	SOD (U/mg)	MDA (nmol/mg)
Control	8.45 ± 0.75	13.78 ± 1.5	2.56 ± 0.28
OVA	3.57 ± 0.35*	6.50 ± 0.9*	8.56 ± 0.75*
ALA + OVA	7.10 ± 0.80$^{\#}$	10.94 ± 1.2$^{\#}$	4.70 ± 0.52$^{\#}$

Values are presented as mean ±SEM. $^{*}P < 0.05$, compared with control; $^{\#}p < 0.05$ compared with OVA

Effect of ALA on protein expressions of TNF-α and NF-κB

The protein expression of cytoplasmic TNF-α and nuclear NF-κb p-p65 (active subunit) in lung homogenates were quantified using immunoblot technique. The protein expressions of TNF-α and NF-κb p-p65 were upregulated in neonatal mice from mother mice sensitized with OVA ($p < 0.05$; Figure 3). However, marked downregulations in the protein expressions of these inflammatory markers (TNF-α and NF-κb p-p65) were observed in ALA-administered neonatal mice, when compared to neonatal mice born from OVA-exposed mice ($p < 0.05$).

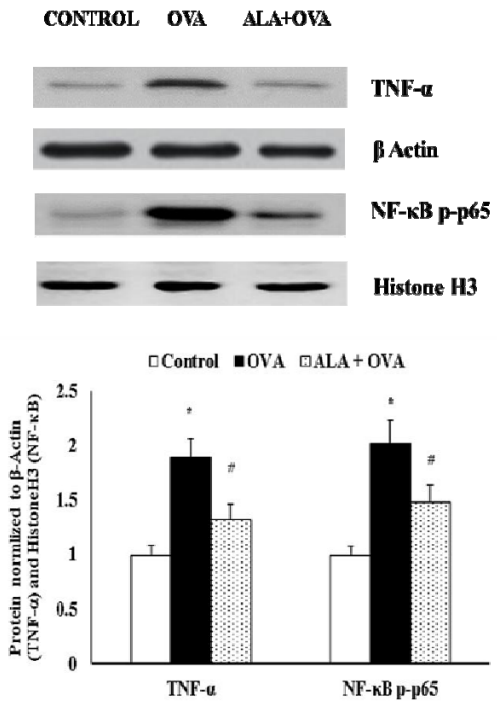

Figure 3: Protein expression of cytoplasmic TNF-α and nuclear NF-κb p65 (active subunit) in lung homogenate of neonatal OVA-exposed mice. Values are presented as mean ± SEM. $^{*}P < 0.05$, compared with control; $^{\#}p < 0.05$, compared with OVA group

DISCUSSION

This is the very first animal study conducted to investigate the protective effect of ALA during pregnancy and lactation, against susceptibility to OVA-induced neonatal asthma in a mouse model, and the involvement of the NF-κB inflammatory signaling pathway in the process. The results of the present animal study showed that neonatal mice from pregnant mice which received 1 % ALA (from 6th day of gestation to 21st day of lactation) had significantly lowered population of inflammatory cells, lower levels of inflammatory markers, lower levels of MDA, reduced levels of OVA-specific IgE and total IgE, and improved antioxidant status. Moreover, the results revealed that the ALA-induced reduction in inflammatory responses occurred through a mechanism involving downregulation of the protein expressions of TNF-α and NF-κb p-p65 which are linked to the NF-κB signaling pathway.

It has been reported that inflammatory cells, especially eosinophils, neutrophils, lymphocytes and macrophages play crucial roles in the induction of asthma due to the fact that they are directly involved in excess mucus formation (accumulation) and airway remodelling [18,19]. Likewise, in this study, mice treated with OVA also showed increased infiltration of eosinophils and other inflammatory cells. However, ALA-treated pups (neonatal mice) had decreased levels of infiltration by eosinophils and other inflammatory cells. These results might be due to the anti-inflammatory effect of ALA, as well as its apoptotic effect on eosinophils [11].

It has been reported that various inflammatory cytokines (IL-5, IL-4, IL-13, and TNF-α) are elevated in asthmatic patients [15]. Thus, the production levels of these Th2 cell-mediated cytokines in OVA-sensitized and ALA-treated neonatal mice were determined in this investigation. The results indicated that the concentrations of these inflammatory were significantly elevated in OVA-induced asthmatic neonatal mice. The increases in concentrations of IL-5, IL-4, IL-13 and TNF-α were significantly reversed in ALA-treated pups (neonatal mice). These data are consistent with the results of Park *at al* who reported that treatment with lipoic acid considerably decreased the levels of these cytokines due to its antioxidant and anti-inflammatory properties. Immunoglobulin E (IgE) is involved in airway inflammation, and it is a crucial mediator of allergic response in asthma patients. It is produced by B cells which are regulated by inflammatory cytokines IL-4 and IL-13. Therefore, many researchers have suggested that any drug that exerts an anti-IgE effect can be used for treating asthma [20,21]. In this study, levels of IgE and OVA-sp IgE were significantly elevated in neonatal mice from OVA-exposed mother mice. In contrast, neonatal pups littered and lactated by mice treated with ALA showed decreased levels of IgE and OVA-sp IgE. Similarly, a study conducted by Nakano and his colleagues [22] demonstrated that lipoic acid derivative (DHL-His Zn) significantly suppressed levels of OVA-specific IgE.

Several studies indicated that elevated oxidative stress is one of the major features of asthmatic patients [23,24]. In this study, the activities of

CAT and SOD were significantly reduced in OVA-sensitized mice, while MDA levels were increased. However, neonatal mice from mother mice treated with ALA had significant improvement in the levels of CAT and SOD, along with suppressed MDA levels. Since ALA shows potent metal ion-chelating activity (due to presence of two sulfur atoms) and free radical scavenging activity (as a result of availability of free OH group,) it can effectively improve antioxidant status in ALA-treated neonatal mice.

It is well documented that the NF-κB signaling pathway is the master regulator (transcription factor) of the inflammatory cascade and immune response in the various models [25,26]. It is known that NF-κB consists of hetero- and homo-dimer complexes of p50 and p65 subunits, with the NF-κB p65 subunit being one of the major bioactive subunits. Upon stimulation with either LPS or OVA, the NF-κB p65 subunit is activated and translocated from the cytoplasm to the nucleus where it triggers on the expressions of the various inflammatory cytokines (IL-1β, IL-5, IL4, IL-6, IL-8, IL-13, IL-17) [17,27]. As stated earlier, these cytokines are major contributors to the etiology of asthma. Thus, any anti-asthmatic agent should regulate the NFκB signaling pathway [28]. It may be reasonably hypothesized that ALA might abolish asthmatic airway inflammatory response (airway remodeling) by downregulating the NF-κB signaling pathway, thereby suppressing the production of TNF-α and subsequently, the production of various inflammatory cytokines (IL-5, IL-4 and IL-13) as well as IgE production, in OVA-induced mice model of asthma.

The protein expressions of TNF-α and NF-κb p-p65 were elevated in neonatal mice sensitized with OVA. This finding is consistent with those of many researchers using OVA animal model [29,30]. It has been reported that ALA-treated mice pups (neonatal mice) showed marked downregulation of protein expressions of TNF-α and NF-κb p-p65. Similarly, Cho and co-workers [13] reported that lipoic acid significantly down-regulated NF-κB binding activity in OVA-induced asthma mouse model. However, Lin and his co-workers [31] demonstrated that alpha-lipoic acid markedly suppressed the protein expression of the NF-κB p-p65 subunit (phosphorylated form), and thus inhibited the activation of LPS-induced acute lung injury. These findings indicate that ALA-treated offspring mice pups (neonatal mice) received the benefits of ALA from mother mice via the placenta and lactation. This resulted in suppression of asthma-induced airway inflammatory response, as was evident in lower inflammatory cell counts, reduced inflammatory cytokines, and decreased levels of OVA-specific IgE and total IgE) via downregulation of the NF-κB signaling pathway.

This study has few limitations. These are the exclusion of parameters related to apoptosis, haematological and blood clotting factors, and histopathology. These parameters will be included in subsequent studies.

CONCLUSION

This study has shown that ALA administration during pregnancy (maternal exposure) abolishes ALA-induced asthmatic airway inflammatory response (airway remodeling) in offspring through downregulation of NF-κB signaling pathway, as well as improvement of antioxidant status. Thus, ALA significantly suppresses airway inflammation/remodeling, and might be useful as an adjuvant therapy to conventional anti-asthmatic drugs for treating asthma patients. However, a large-scale clinical study is needed to confirm this conclusion.

DECLARATIONS

Acknowledgement

This study was financially supported by Taihe Hospital, Hubei University of Medicine.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

Both Yong Jiang and Xingjuan Liao contributed equally. Yong Jiang, Didi Tao and Xingjuan Liao involved in conducting and concepting this study. Shengsheng Wang, Zhifu Ni, and Rui Liang helps in statistical analysis. Yuling Zhang and Didi Tao involved in maintaining of animals. Yong Jiang, Didi Tao and Xingjuan Liao drafted this manuscript.

REFERENCES

1. Bostantzoglou C, Delimpoura V, Samitas K, Zervas E, Kanniess F, Gaga M. Clinical asthma phenotypes in the real world: opportunities and challenges. Breathe 2015; 11: 186-193. https://doi.org/10.1183/20734735.008115
2. Walker SM, Akdis C, Dahlen SE, Djukanovic R, Edwards J, Garcia-Marcos L, Johnston S, Kupczyk M, Martin T, Myles D, et al. Building the investment case for asthma R & D: the European Asthma Research and Innovation Partnership argument. Clin Exp Allergy 2016; 46: 1136-1138.
3. Fahy JV. Type 2 inflammation in asthma-present in most, absent in many. Nat Rev Immunol 2015; 15: 57.
4. Xing X, Wang H. Anti-asthmatic effect of laurotetanine extracted from Litsea cubeba (Lour.) Pers. root on ovalbumin-induced allergic asthma rats, and elucidation of its mechanism of action. Trop J Pharm Res 2019; 18: 1277-1283.
5. Fei X, Zhang X, Zhang GQ, Bao WP, Zhang YY, Zhang M, Zhou X. Cordycepin inhibits airway remodeling in a rat model of chronic asthma. Biomed Pharmacother 2017; 88: 335-341.
6. Kumar D, Bhujbal SS, Patil PS, Buge PV. In vitro and in vivo activities of stem bark of methanolic extract of Ailanthus excelsa Roxb. in the management of asthma. Int J Pharmacol 2010; 6: 284-289.
7. Maltby S, Gibson PG, Powell H, McDonald VM. Omalizumab treatment response in a population with severe allergic asthma and overlapping COPD. Chest 2017; 151: 78-89.
8. Bosnjak B, Stelzmueller B, Erb KJ, Epstein MM. Treatment of allergic asthma: modulation of Th2 cells and their responses. Respir Res 2011; 12: 114.
9. Rochette L, Ghibu S, Richard C, Zeller M, Cottin Y, Vergely C. Direct and indirect antioxidant properties of α-lipoic acid and therapeutic potential. Mol Nutr Food Res 2013; 57: 114-125.
10. Mei XH, Yang YW. Neuroprotective effects of α-lipoic acid against hypoxic–ischemic brain injury in neonatal rats. Trop J Pharm Res 2017; 16(5): 1051-1058.
11. Zhang Y, Han P, Wu N, He B, Lu Y, Li S, Liu Y, Zhao S, Liu L, Li Y. Amelioration of lipid abnormalities by α-lipoic acid through antioxidative and anti-inflammatory effects. Obesity 2011; 19: 1647-1653.
12. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. Biochim Biophys Acta (BBA) 2009; 1790: 1149-1160.
13. Cho YS, Lee J, Lee TH, Lee EY, Lee KU, Park JY, Moon HB. α-Lipoic acid inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. J Allergy Clin Immunol 2004; 114: 429-435.
14. Park SJ, Lee KS, Lee SJ, Kim SR, Park SY, Jeon MS, Lee HB, Lee YC. L-2-Oxothiazolidine-4-carboxylic acid or α-lipoic acid attenuates airway remodeling: involvement of nuclear factor-κB (NF-κB), nuclear factor erythroid 2p45-related factor-2 (Nrf2), and hypoxia-inducible factor (HIF). Int J Mol Sci 2012; 13: 7915-7937.
15. Dong F, Wang C, Duan J, Zhang W, Xiang D, Li M. Puerarin attenuates ovalbumin-induced lung inflammation and hemostatic unbalance in rat asthma model. Evid Based Complement Alternat Med 2014; 2014.
16. Hsu DZ, Liu CT, Chu PY, Li YH, Periasamy S, Liu MY. Sesame oil attenuates ovalbumin-induced pulmonary edema and bronchial neutrophilic inflammation in mice. BioMed Res Int 2013; 2013
17. Li Z, Zheng J, Zhang N, Li C. Berberine improves airway inflammation and inhibits NF-κB signaling pathway in an ovalbumin-induced rat model of asthma. J Asthma 2016; 53: 999-1005.
18. Zhu S, Wang H, Zhang J, Yu C, Liu C, Sun H, Wu Y, Wang Y, Lin X. Anti-asthmatic activity of quercetin glycosides in neonatal asthmatic rats. 3 Biotech 2019; 9(5): 189.
19. Kandhare AD, Bodhankar SL, Singh V, Mohan V, Thakurdesai PA. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. Biomed Aging Pathol 2013; 3(1): 23-30.
20. Kuhl K, Hanania NA. Targeting IgE in asthma. Curr Opin Pulm Med 2012; 18: 1-5.
21. Buhl R. Anti-IgE antibodies for the treatment of asthma. Curr Opin Pulm Med 2005; 11: 27-34.
22. Nakano T, Hsu LW, Lai CY, Takaoka Y, Inomata M, Kitano S, Chen CL, Goto S. Therapeutic potential of α-lipoic acid derivative, sodium zinc histidine dithiooctanamide, in a mouse model of allergic rhinitis. Int Forum Allergy Rhinol 2017; 7: 1095-1103.
23. Nadeem A, Chhabra SK, Masood A, Raj HG. Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol 2003; 111: 72-8.
24. Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci Ö. Oxidative stress in asthma: Part of the puzzle. Pediatr Allergy Immunol 2018; 29: 789-800.
25. Taniguchi K, and Karin M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol 2018; 18: 309-324.
26. Wulczyn FG, Krappmann D, Scheidereit C. The NF-κB/Rel and IκB gene families: mediators of immune response and inflammation. J Mol Med 1996; 74: 749-69.
27. Zhang T, Yang S, Du J, Jinfu Y, Shumin W. Platycodin D attenuates airway inflammation in a mouse model of allergic asthma by regulation NF-κB pathway. Inflammation 2015; 38: 1221-1228.
28. Peng S, Gao J, Liu W, Jiang C, Yang X, Sun Y, Guo W, Xu Q. Andrographolide ameliorates OVA-induced lung injury in mice by suppressing ROS-mediated NF-κB

signaling and NLRP3 inflammasome activation. Oncotarget 2016; 7(49): 80262.

29. *Yan GH, Choi YH. Phellinus linteus extract exerts anti-asthmatic effects by suppressing NF-κB and p38 MAPK activity in an OVA-induced mouse model of asthma. Immune Network 2014; 14: 107-115.*
30. *Zhou E, Fu Y, Wei Z, Yang Z. Inhibition of allergic airway inflammation through the blockage of NF-κB activation by ellagic acid in an ovalbumin-induced mouse asthma model. Food Funct 2014; 5: 2106-2112.*
31. *Lin YC, Lai YS, Chou TC. The protective effect of alpha-lipoic acid in lipopolysaccharide-induced acute lung injury is mediated by heme oxygenase-1. Evid Based Complement Alternat Med 2013; 2013. https://doi.org/10.1155/2013/590363*