The Study on the Molecular Mechanism of Erchen Tang on Asthma Based on Network Pharmacology

Yuzhe Ren
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital
Haijing Zhang
Yike(Beijing) Pharmaceutical Technology Co., Ltd
Zhou Yu
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital
Jun Wan
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital
Xuejin Peng
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital
Yilin Huang
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital
Chengke Li
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital
Xiangzheng Yang (ZYEKYXZ@163.com)
Beijing University of Chinese Medicine Affiliated Shenzhen Hospital

Research

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Abstract

Objective: To explore the active ingredients of ECT and their targets of asthma and investigate the potential mechanism of ECT on asthma.

Methods: Firstly, the active ingredients and target of ECT were screened for BATMAN and TCMSP, functional analysis was finished via DAVID. Then, animal model was induced by ovalbumin (OVA) and aluminum hydroxide. Eosinophil (EOS) counts, EOS active substance Eosinophilic cationic protein (ECP) and eotaxin levels were detected followed the instruction. Pathological changes of lungs tissue were examined by H&E staining and transmission electron microscopy. Interleukin (IL-4, IL-10, IL-13, TNF-α), TIgE and IgE level in bronchoalveolar lavage fluid (BALF) were measured by ELISA. Finally, the protein expression of TGF-β / STAT3 pathway to lung tissue was detected by Western Blot.

Results: A total of 450 compounds and 526 target genes were retrieved in Erchen tang. Functional analysis indicated that its treatment of asthma was associated with inflammatory factor and fibrosis. In the animal experiment, the results showed that ECT significantly regulated inflammatory cytokine (IL-4, IL-10, IL-13, TNF-α) levels in (P<0.05, P<0.01) , reduced EOS number (P<0.05) and also ECP and Eotaxin levels in blood (P<0.05) in BALF and / or plasma. Bronchial tissue injury was obviously improved on ECT treatment. Associated protein in TGF-β / STAT3 pathway were significantly regulated by ECT (P<0.05).

Conclusion: This study originally provided the evidence that the Erchen tang was effective against the treatment of asthma symptoms, and its underlying mechanism might be regulation of inflammatory factor secretion and TGF-β/STAT3 signaling pathway.

1. Background

Asthma is a common disorder characterized by airway inflammation and by declining in lung function with airway remodeling in a subset of asthmatics[1]. Related studies have determined that the inflammation of asthma is exacerbated by Th2 activity[2]. Activated Th2 cells secrete more inflammatory cytokines such as IL-4 and IL-13, thus causing airway hyperresponsiveness (AHR), eosinophil infiltration, goblet cell hyperplasia, and excessive mucus secretion[3]. IL-13 and IL-4 partly share the same receptor and signaling pathways and both are deeply involved in IgE synthesis, eosinophil activation, mucus secretion and airways remodeling[4]. Furthermore, in airway remodeling, TGF-β / STAT3 pathway plays an important role, in which JAK activated by TGF-β and cause the up-regulated STAT3 level[5], which was already known that both TGF-β and STAT3 were tightly related to airway fibrosis[6, 7]. Present treatment of Cough Variant Asthma (CVA) patients with bronchodilators, glucocorticoids and leukotriene receptor antagonists cannot fundamentally alleviate chronic inflammation of the airway[8, 9]. Therefore, it is urgent to develop drugs with significant therapeutic effects on asthma by regulating the TGF-β / STAT3 pathway to suppress airway inflammation.

Recent studies indicated that extracts from Chinese medicine or other herbs have immune regulation function[10] and good effect on the treatment of asthma[11]. Erchen tang (ECT), as a famous

prescription of traditional Chinese medicine first collected in ancient Chinese medicine book 'Taiping Huimin Heji Ju Fang', was included in Chinese pharmacopoeia[12], has achieved good results such as less coughing and less sputum in clinical treatment of various types of bronchial asthma [13, 14]. In laboratory studies, the therapeutic effects of drugs on asthma are usually studied on animal model induced by ovalbumin (OVA) and aluminum hydroxide[15], and rats are often used in traditional Chinese medicine pharmacodynamics study as asthma models[16], [17]. It was proved by animal experiments that ECT could regulate the cytokine levels (such as IL-1, IL-6 and TNF-α) in serum[18] and prevent the airway remodeling by inhibit the expression of MMP-9 in lung tissue on asthma rats induced by ovalbumin (OVA) and aluminum hydroxide[19], and its improvement in lung tissue and the pulmonary function of chronic bronchitis model rats was also proved by Gao Miaoran[20]. However, there are still lots of unclear points of ECT, such as its active ingredients and the specific target of the active ingredient, to solve these problems, we further used network pharmacology to lucubrate the active ingredients and the mechanisms of ECT.

Network pharmacology (NP) is a useful discipline that emphasizes maximizing drug efficacy and minimizing adverse effect via the multiple regulation of the signaling pathway[21]. It has been well applied to the study of the mechanisms of traditional Chinese medicine (TCM)[22] for almost all TCM and worldwide ethnomedicine exert therapeutic effect by targeting multiple molecules of the human body. Such as prediction of potential targets and pathways of cordycepin on the proliferation of MCF-7 breast cancer cells[23] and screen of active compounds of Curcumae Rhizoma[24]. Since the active ingredients and mechanism of ECT in treating asthma is not clear yet, NP is an effective way to create the targets-pathways interaction, and the targets related to asthma was collected to create pathways-disease interactions, as well as further related bioinformatics analysis, was employed to explain the potential mechanism of treatment of ECT on asthma.

In this study, we used network pharmacology to explore the active ingredients of ECT and their targets of asthma. Thereby, the effect of Erchen Tang on asthma and the potential molecular mechanisms of TGF-β/STAT signaling pathway were studied by asthma animal experiments.

1. Methods

1.1 Website database

TCMSP database (http://lsp.nwu.edu.cn/index.php);

BATMAN (http://bionet.ncpsb.org/batman-tcm/);

Metascape (http://metascape.org/gp/index.html#/main/step1);

DAVID (https://david.ncifcrf.gov/);

KEGG (https://www.kegg.jp/).

1.2 Animals and agents

1.2.1 Animals

Forty male Wistar rats, 200 ± 20 g, purchased from Beijing Weitong Lihua Experimental Animal Co., Ltd., the animal certification number is: 11400700256012. Rats were free of pathogens and respiratory disease, and used in accordance with guidelines set forth by the All-University Committee on Animal Use and Care (Animal ethics committee approval number: [SCXK (Beijing) 2016-0006]). All animal experiments were performed at Beijing University of Chinese Medicine Affiliated Shenzhen Hospital. Animals were housed in an environment with temperature of 20 ~ 25℃, relative humidity of 40 ~ 70%. After the experiment, the rats were euthanized by intravenous injection of 0.3% sodium pentobarbital.

1.2.2 Agents

Erchen tang was provided by the First Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine (Lot number: 20150102), the ratio of each medicine in the prescription is *Pinellia ternata* (Thunb.) Makino: *Citrus reticulata* Blanco: *Smilax glabra* Roxb.: *Glycyrrhiza aspera* Pall.=10:10:6:3; 4% paraformaldehyde was bought from Beijing Suo Laibao Technology Co., Ltd.(Beijing, China); OVA sIgE enzyme-linked immunosorbent kit was bought from Xiamen Huijia Biological Technology Co., Ltd. (Xiamen, China); Ulatan was bought from Wuhan Hongxinkang Fine Chemical Co., Ltd.(Wuhan, China); Sodium chloride injection was bought from Shandong Hualu Pharmaceutical Co., Ltd.(Shandong, China); PVDF membrane was bought from Millipore Corporation of the United States(United States); EasySee Western Marker was bought from Beijing Quanjin Biotechnology Co., Ltd.(Beijing, China); Real-time PCR Kit was bought from Swiss Roche(Swiss); Trizol reagent was bought from American Invitrogen(America). Ovalbumin, Aluminum hydroxide, ECP enzyme immunoassay kit, IgE enzyme immunoassay kit, Eotaxin Elisa kit were all bought from Sigma (America). Horseradish peroxidase-conjugated goat anti-rabbit/anti-mouse/anti-goat antibody, Tissue protein extraction reagent, BCA Protein Quantitation Kit, SDS-loading Buffer, SDS-PAGE gel preparation kit, High sensitivity chemiluminescence detection kit and cDNA reverse transcription kit was all bought from Beijing Quanjin Biotechnology Co., Ltd. (Beijing, China).

1.3 Target gene screening and Functional enrichment analysis

Erchen tang was constituted by *Pinellia ternata* (Thunb.) Makino, *Smilax glabra* Roxb., *Glycyrrhiza aspera* Pall., and *Citrus reticulata* Blanco. Firstly, we separately queried the chemical composition of these four Chinese herbal medicines from the database of BATMAN and TCMSP. Then we combined the compounds of each medicinal material and imported them into TCMSP to get their target genes. We further normalized these genes and got human related genes in database of Metascape. Finally, we used DAVID to perform functional enrichment analysis of human related genes, the mechanism map was obtained from KEGG.

1.4 Animal experiment

1.4.1 Animal model of asthma

One week after adaptive feeding, except for the normal control group, other groups were nihilated of ovalbumin (OVA) and aluminum hydroxide: on the 0th and 12th day of the experiment, each rat was intraperitoneally injected 1 mL of 10% OVA solution (containing sterile antigen solution of Class II OVA 1 mg and 100 mg of aluminum hydroxide gel) of sensitization, and then challenged with 5% OVA for 30 min every day for 5 days, then every other day, for 6 weeks.

1.4.2 Grouping and administration

Eighty rats were randomly divided into 4 groups, normal-controlled group, model-controlled group, positive controlled group (dexamethasone, DXMS, 1 mg/kg), Erchen tang group (4.5 g/kg) individually, the ratio of each medicine in ECT is *Pinellia ternata* (Thunb.) Makino: *Citrus reticulata* Blanco: *Smilax glabra* Roxb.: *Glycyrrhiza aspera* Pall. = 10:10:6:3).

1.4.3 Experimental material

24 h after last injection, 20% urethane solution was used for anesthetizing by intraperitoneal injection. Blood was taken from the abdominal aorta, partially placed in the EDTA anticoagulation tube, and the rest of the blood was placed in a non-anticoagulant tube, 3000 r/min, centrifuged for 15 min and collected the serum. The blood vessels of the neck were separated from the nerves, and the main bronchus was separated, the left bronchus was ligated, the right pulmonary alveoli through 1 cm below the main bronchus was rinsed by injecting 0.6 mL 37% isotonic sodium chloride solution each time for three times. Total 1.35 ~ 1.55 mL liquid (bronchoalveolar lavage fluid, BALF) was collected, the supernatant wad used to detect indicators such as IL-4 and IL-13. One part of the right lung was fixed with 4% paraformaldehyde, and the other part of the right lung was frozen in liquid nitrogen. Take a suitable amount of cryopreserved lung tissue, and 9 times of low-temperature refrigerated physiological saline was added to prepare 10% tissue homogenate.

1.4.4 Histopathology and tissue imaging

After dehydration, embedding and Sect. 4 ~ 5 μm of tissue, hematoxylin staining was carried out for 6 ~ 10 min. The mixture was separated by 1% hydrochloric acid solution, diluted ammonia water was used for 10 min, then eosin staining for 10 s. Tissue inflammation was observed after fixation. Tissue imaging was observed after 3% uranyl acetate-lead citrate dyed. The computerized image analyzer (Pathological image analyzer, Lycra, Japan) was used to measure the bronchial lumen and outer circumference (Pi, Pe), the number of bronchial smooth muscle cells (Pi). Calculating the basement membrane area (WAi), airway smooth muscle layers area (WAm), and make WAi, WAm and the ratio of Pi was measured as a standardization, representing bronchial basement membrane thickness (WAi/Pi) and smooth muscle layer thickness (WAm/Pi), respectively.

1.4.5 Eosinophil count, EOS active substance ECP and Eotaxin levels

BALF was centrifuged at 2000 rpm for 5 min (Benchtop centrifuge, Shanghai Anting Scientific Chemical Instrument Factory), the supernatant of it was discarded, and the cell sediment was diluted in 1 mL of

pre-cooled PBS. The precipitate of BALF was resuspended for total cell counts recorded with on a hemocytometer. Slide were prepared by using a cytospin instrument. At least 500 inflammatory cells were counted in each sample. Total EOS number per microscope field was determined by counting EOS under an optical microscope (Microplate scanning microplate reader American BioTek instrument). Then the absolute EOS number was determined. EOS active substance ECP and Eotaxin in blood were detected by Elisa Kit according to the manufacture instructions.

1.4.6 Enzyme-Linked Immunosorbent Assay (ELISA)

Interleukin (IL-4, IL-10, IL-13, TNF-α), TIgE and IgE level in serum were measured by ELISA using monoclonal antibody-based mouse interleukin ELISA kit according to the manufacturer's instruction. All data represent the mean and standard error of mean from at least three separate experiments and were compare by analysis of variance (ANOVA).

1.4.7 Detection of the expression of TGF-β/STAT3 signaling pathway

The expression of TGF-β\STAT3 signaling pathway in BALF was detected by western blot (Bio-Rad electrophoresis system, Bole Life Medical Products Co., Ltd.). Western blot analysis was performed according to the instruction. Briefly, lung homogenate was centrifuged in 5000 r/min for 5 min, sedimentary tissue was collected and lysed on ice in a RIPA buffer with protease inhibitor 1% PMSF, and then clarified by centrifugation. Total cell lysates were resuspended in SDS sample buffer and resolved by SDS-PAGE. Proteins were transferred to PVDF membrane and then blocked with 5% BSA / TBST buffer for 1 h at room temperature. The blocked PVDF membrane was directly placed in primary antibody solution and incubated overnight at 4 ℃ and then transferred to second antibody solution and incubated at room temperature for 2 h. After that was color development, the gel imaging system (Gel imaging system, American Bio-RAD company) was used to scan and film. All experiments were performed in triplicate.

The collected BALF was centrifuged, and the supernatant was collected. The RPMI 1640 culture solution was resuspended,the cell sediment was diluted and cultured at 37 °C in a 5% CO_2 incubator for 2 h to extract macrophages and then lymphocytes were removed. Rinsing with PBS solution (containing 10% FBS), centrifuging, and further separating with precooling separation solution. Finally, interface cells of 1.105 to 1.090 were collected and rinsed with PBS (containing 10% FBS). Separated EOS was adjusted to a cell concentration of 2×10^5 with RPMI 1640 complete medium containing 10% FBS, and 10 U/ML IL-5 cytokine was added, and cultured in a culture plate at 5% CO_2 and 37 ° C. After 24 h, the total protein was extracted and quantified, and the proteins related to TGF-β\STAT3 signaling pathways were detected. The detection method was the same as the previous one. It should be noted that the protein extraction reagent was the mammalian protein extraction reagent (containing 1% PMSF). Morphological identification of separated EOS above was observed by Wright-Giems staining on room temperature with Giems dye liquor. After washing, first rinse the slide with PBS solution, then rinse with small stream of water, seal and observe.

1.5 Statistical analysis

Data are presented as the mean ± standard deviations (SD) and Student's t-test combined with one-way ANOVA previously was performed to determine statistically significant differences between two groups. Results were considered statistically significant at *p* values 0.05(#), 0.01(##), or 0.001(###) for the control group *versus* the model group comparisons and *p* values 0.05(*), 0.01(**), or 0.001(***) for the model group *versus* the experimental group.

2. Results

2.1 Analyzing the potential mechanism of ECT in the treatment of asthma by network pharmacology

The result of database search showed that a total of 450 compounds and 526 target genes were retrieved in Erchen tang, and 98 genes closely related to asthma among them (Supplementary material 1). Functional enrichment indicated that ECT could regulate asthma (Supplementary material 2). The underlying mechanism was shown in Fig. 1, it could affect the secretion of IL-4, IL-13, TNF-α, eotaxin and so on, thereby inhibited the increase of a cells, and finally achieved regulation of tracheal inflammation and remodeling (Fig. 1).

2.2 Effect of ECT on inflammatory cytokine levels in BALF

To evaluate the degree of inflammation, cytokines (IL-4, IL-10, IL-13, TNF-α) levels in BALF were detected by Elisa kit according to manufacture introduction. Our data (Fig. 2) showed that, compare with model group, significantly reduction of IL-13 and TNF-α concentrations from ECT group were observed ($P < 0.05$), and significantly increased levels of IL-4, IL-10 from ECT group were observed ($P < 0.05$, $P < 0.01$). In the model group, IL-4, IL-10 levels (Fig. 2A/ 2B) had significantly decreased ($P < 0.05$, $P < 0.001$), and IL-13, TNF-α levels (Fig. 2C/2D) had significantly increased, compare with control group ($P < 0.001$). These data suggested that ECT could regulate cytokine levels from T cells and macrophage like IL-4, IL-10, IL-13 and TNF-α.

2.3 Effect of ECT on serum TIgE and OVA sIgE levels

Results from Fig. 3 showed that the levels of TIgE (Fig. 3A) and OVA sIgE (Fig. 3B) in the model group were significantly higher than those in the control group ($P < 0.001$). Compare with the model group, dexamethasone and Erchen tang treatment significantly decrease the expression levels of TIgE and OVA sIgE ($P < 0.01$).

2.4 Effect of ECT on histopathology of bronchial tissue

HE staining of lung tissue (Fig. 4) showed that the bronchial epithelium of the control group was intact (Fig. 3D), there was no obvious inflammatory infiltration around, the lung tissue and the area of bronchial were normal. The lumen of the model group was narrowed, and many inflammatory cells infiltrated around the blood vessels and lung tissues, neutrophils and eosinophils were main types of them. Also, for

model group, the basement membrane was thickened and irregularly shaped, and the connective tissue of the smooth muscle and bronchus of the wall was obviously proliferated. Compare with model group, the inflammatory infiltration of the dexamethasone group and the ECT group was significantly alleviated, the damage caused by the above model was significantly improved.

Statistical analysis showed that the ratios of WAi/Pi (Fig. 3A), WAm/Pi (Fig. 3B) and N/Pi (Fig. 3C) of model group were significantly higher than those in the control group ($P < 0.01$, $P < 0.001$). Both dexamethasone and ECT treated group significantly reduced the ratio of WAi/Pi, WAm/Pi and N/Pi ($P < 0.05$, $P < 0.01$).

2.5 Effect of ECT on pulmonary imaging

The ultra-microstructure of lung tissue (Fig. 5) was observed by transmission electron microscopy. The internal structure of the control group was normal, the mitochondria structure and basement membrane structure were intact, the sputum was clear with many visible lamellar bodies. For model group, EOS cells number increased significantly, the mitochondria were swollen and the sputum was blurred, also the number of lamellar corpuscles decreased significantly, and the number of voids increased with chromatin accumulated. With ECT treated, the basement membrane of ECT group is intact, and eosinophil aggregation with chromatin accumulated were both reduced compare with model group.

2.6 Effect of ECT on Eosinophil count and Tissue EOS activation markers' levels

Our data (Fig. 6) showed that marked EOS count was observed in BALF and plasma from asthma model group. Compare with control group, ECP and Eotaxin levels of model group were significantly increased ($P < 0.05$, $P < 0.001$) both in BALF (Fig. 6B/ 6C) and plasma (Fig. 6D/ 6E). The number of EOS in BALF and plasma both reduced significantly compare with model group with ECT treatment ($P < 0.05$). Also, ECP and Eotaxin were EOS activation markers in lung tissue. ECP and Eotaxin levels were significantly decreased compare with model group with ECT treatment ($P < 0.05$). These data suggested that ECT could reduce EOS number both in BALF and plasma, also ECP and Eotaxin levels in BALF, which is possible mechanism for asthma treatment.

The EOS cells separated from BALF were identified by Wright-Giemsa and observed under microscope. The morphology and coloration of the cells were consistent. The Wright staining results was positive (Fig. 6A), which proved that the separated cells were the EOS cells. For EOS cells, purity testing showed that each group had EOS purity > 95%.

2.7 Effect of ECT on TGF-β/STAT3 signaling pathway

The protein expression of TGF-β/STAT3 pathway both in lung tissue and separated EOS cells (Fig. 7) were detected by Western Blot. Our data showed that compare with control group, TGF-β, p-STAT3/STAT3 and α-SMA protein levels was significantly up-regulated ($P < 0.01$, $P < 0.001$) both in BALF

(Fig. 7B/7C/7D) and separated EOS cells (Fig. 7F/7G/7H). After treatment of ECT, expression levels of above proteins were decreased significantly ($P < 0.05$).

3. Discussion

Erchen Tang was constituted by *Pinellia ternata* (Thunb.) Makino, *Smilax glabra* Roxb., *Glycyrrhiza aspera* Pall., and *Citrus reticulata* Blanco. *Pinellia ternata* (Thunb.) Makino has protective effects against allergic airway inflammation in a model of asthma in mice[25]. The data supported that *Smilax glabra* Roxb. had immunomodulatory potential through activating macrophages and enhancing host immune system function[26]. Many reports have shown that *Glycyrrhiza aspera* Pall possess anti-inflammatory activity, suppress proinflammatory cytokine like TGF-β and inhibit the translocation of toll-like receptor 4. *Citrus reticulata* Blanco was suggested to be used as a therapeutic agent for patients with Th2-mediated or histamine-mediated allergic asthma[27]. Erchen decoction combined with Yu-Ping-Feng-San has been widely used for decades in treating asthma at the Affiliated Hospital of Nanjing University of Chinese Medicine[28].

Asthma is an immune allergic disease characterized by reversible airway obstruction, airway hyperresponsiveness and airway inflammation[29], among which airway inflammation is the most important pathological change[30] and determines the degree of airway obstruction[31] and airway hyperresponsiveness[32]. It was proved in our study that model group had sever inflammation of bronchus with results from histopathology. Many inflammatory cells infiltrated around the blood vessels and lung tissues. And all these bronchial tissue injuries were improved with ECT treatment, the inflammatory infiltration of the ECT group was significantly alleviated.

Inflammatory cytokines IL-4 and IL-13 has been shown to play a vital role in allergic airway inflammation[33], which promote the airway epithelial cells to release eosinophil chemotactic factor-3 (Eotaxin-3), and then cause airway inflammation of eosinophilia[34]. At the same time, the increased lung LTs mediated by eosinophil, further induced increased IL-13[35], which were secreted by airway epithelial cells, finally caused collagen deposition (i.e., pulmonary fibrosis) mice with respiratory airway inflammation. Our data suggested that the IL-4 and IL-10 levels decreased in asthma model, since IL-10 has been shown to suppress all the pro-inflammatory cytokines, lower IL-10 levels were reported to be associated with higher frequency of bronchial asthma[36], there was result showed significantly increased serum level of IL-10 in children with treatment[37]. ECT could significantly regulate serum inflammatory cytokine (IL-4, IL-10, IL-13, TNF-α) levels, with reduced EOS number both in BALF and plasma, which proved that EOS was related to the regulation of the internal environment balance-maintenance regulation induced by the inflammatory pathway in tissues[38].

It was found that both TGF-β level and pSTAT3/STAT3 would significantly increase either in Bronchial mucosal tissue[39] of asthma patients or in asthma mice[40], and these two proteins are both directly related to tissue fibrosis[41]. It had been indicated that TGF-β signaling was the master pathway regulating fibrosis pathogenesis, in which activator of STAT3 acted as the integrator of various pro-

fibrosis signals[42]. Results from western-blot suggested the growing levels of protein in TGF-β/STAT3 pathway were inhibited with the ECT treatment. Since TGF-β induces integrin, matrix metalloproteinases, protease inhibitors, and regulators of small GTPases that participate in tissue remodeling and influence cell-ECM interactions[43], the regulation of TGF-β/STAT3 pathway might be the mechanism of ECT on airway fibrosis.

4. Conclusions

In this study, network pharmacology was used to predict the potential mechanism of ECT in the treatment of asthma. And the results from the animal experiment indicates that ECT might have modulatory action on cytokines levels (IL-4, IL-10, IL-13, TNF-α) by regulating TGF-β and STAT3 levels, and thereby inhibit the increase of EOS cells, finally achieved regulation of tracheal inflammation and airway fibrosis.

List Of Abbreviation

OVA, Ovalbumin; EOS, Eosinophil; IL-4, Interleukin-4; IL-10, Interleukin-10; IL-13, Interleukin-13; TNF-α, Tumor Necrosis Factor-α; IgE, Immunoglobulin E; TIgE, Total Immunoglobulin E; BALF, Bronchoalveolar Lavage Fluid; ECP, Eosinophilic Cationic Protein; TGF-β, Transforming Growth Factor-β; STAT3, Signal Transducers And Activators Of Transcription-3.

Declarations

Ethics approval and consent to participate

The experimental protocol was approved by the Research Ethics Committee of Beijing University of Traditional Chinese Medicine, China. All participants provided written informed consent

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

Experimental design: XY, YR, ZY, JW, XP. Experimental operation: YR. Analysis and manuscript preparation: HZ, YR. Data analyses: YR, YH and CL.

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Figures

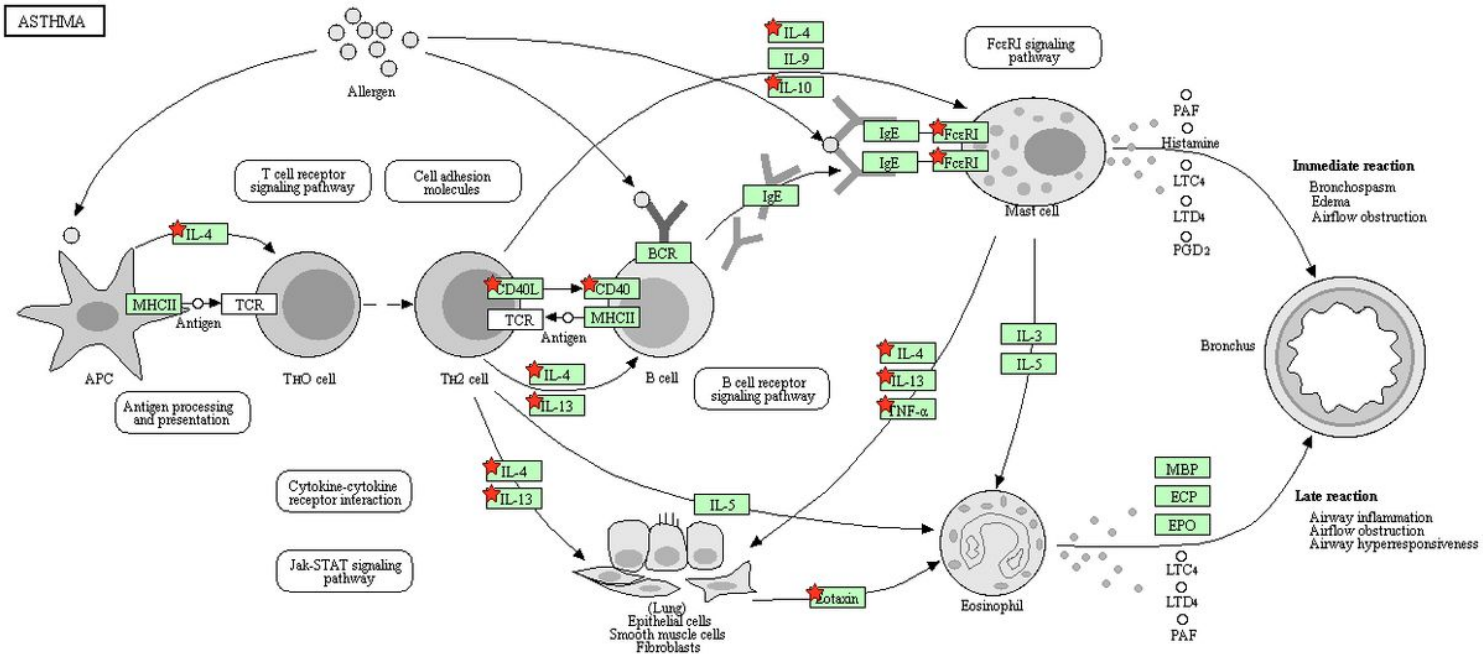

Figure 1

The potential mechanism of ECT in the treatment of asthma. Red five-pointed star represented the target of ECT regulation

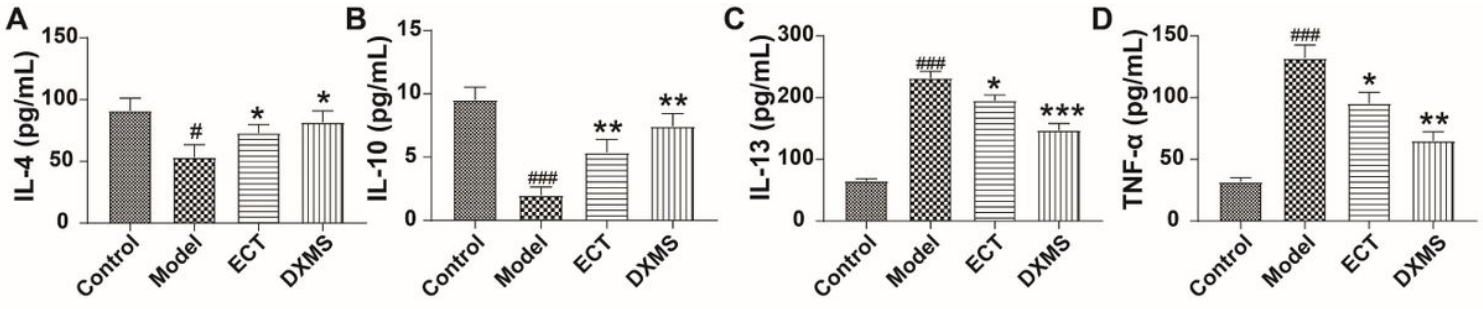

Figure 2

Serum inflammatory cytokine levels of rats (A): represents the IL-4 level in BALF; (B): represents the IL-10 level in BALF; (C): represents the IL-13 level in BALF; (D): represents the TNF-α level in BALF. Data are expressed as mean±SEM (n=8). (Control: normal-controlled group; Model: model-controlled group; ECT: ECT treated group; DXMS: positive-controlled group.). Significant differences #P<0.05, ##P<0.01, ###P<0.001, compare with control group; *P<0.05, **P<0.01, ***P<0.001, compare with model group.

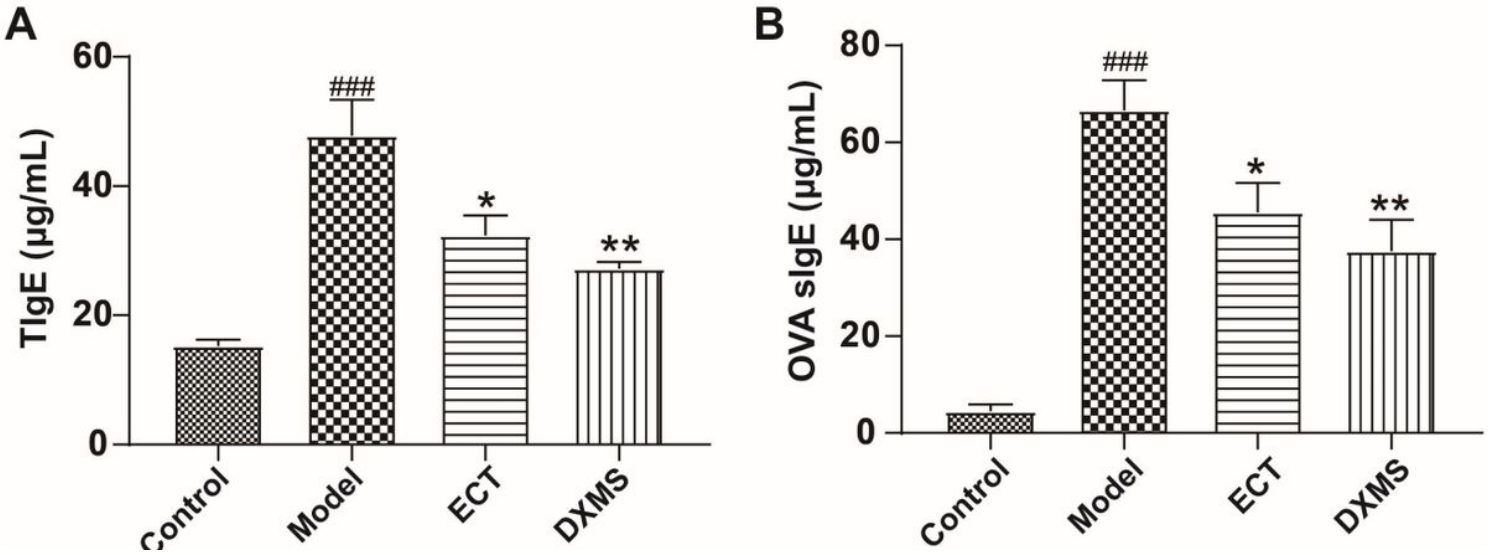

Figure 3

Serum TIgE and OVA sIgE levels of rats (A): represents the TIgE level in serum; (B): represents the OVA sIgE level in serum. Data are expressed as mean±SEM (n=8). (Control: normal-controlled group; Model: model-controlled group; ECT: ECT treated group; DXMS: positive-controlled group.). Significant differences #P<0.05, ##P<0.01, ###P<0.001, compare with control group; *P<0.05, **P<0.01, ***P<0.001, compare with model group.

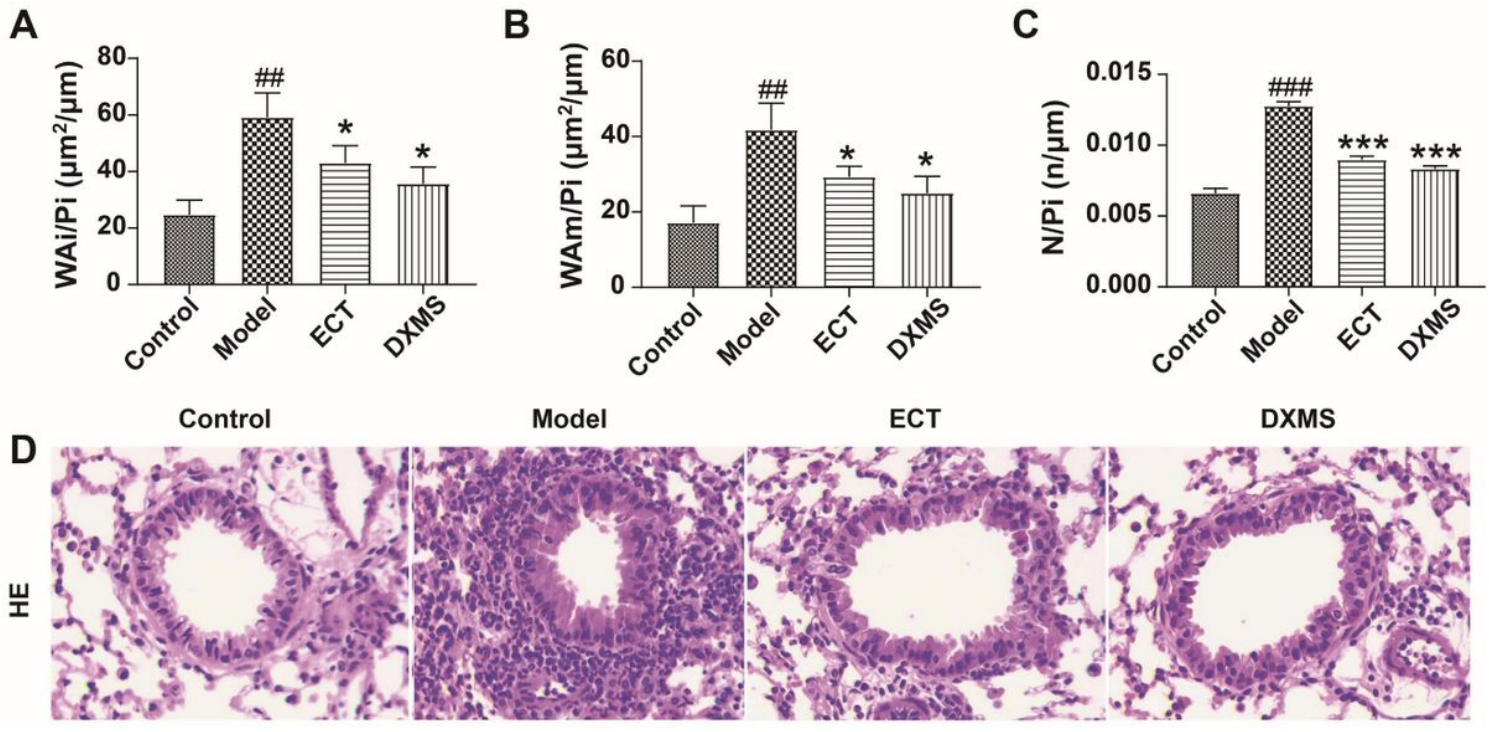

Figure 4

Histopathology of bronchial tissue (A): represents the WAi/Pi value of bronchial; (B): represents the WAm/Pi value of bronchial; (C): represents the N/Pi value of bronchial; (D): represents the HE staining result of bronchial. Data are expressed as mean±SEM (n=8). (Control: normal-controlled group; Model:

model-controlled group; ECT: ECT treated group; DXMS: positive-controlled group.). Significant differences #P<0.05, ##P<0.01, ###P<0.001, compare with control group; *P<0.05, **P<0.01, ***P<0.001, compare with model group.

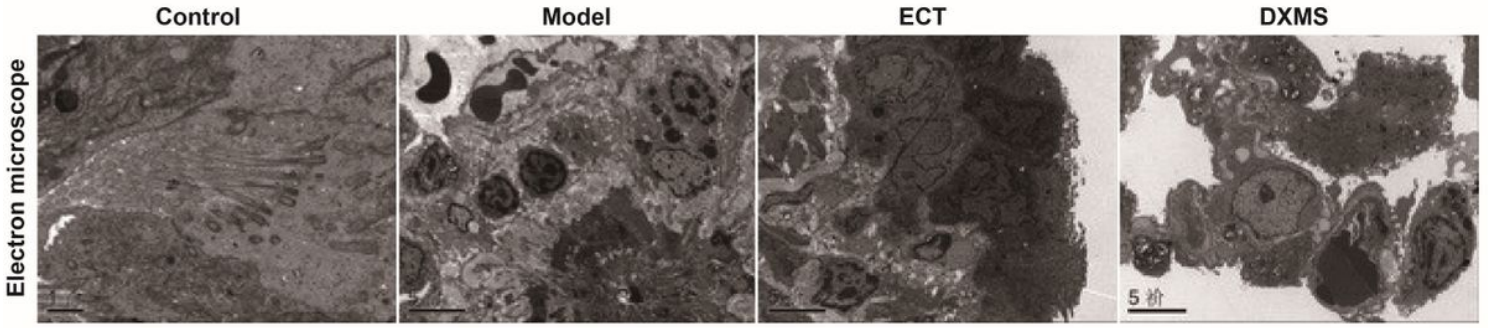

Figure 5

Pulmonary imaging of rats Control: normal-controlled group; Model: model-controlled group; ECT: ECT treated group; DXMS: positive-controlled group.

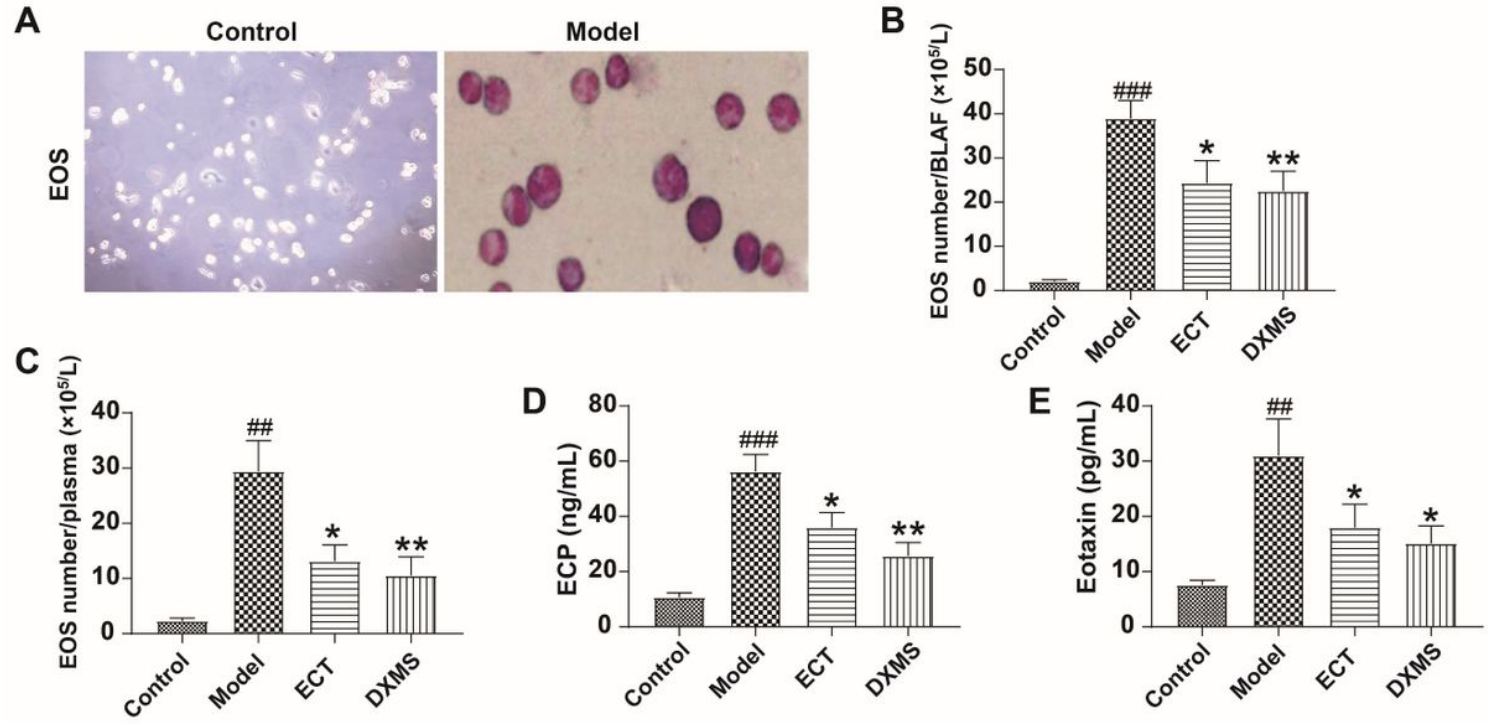

Figure 6

EOS count in BALF and plasma, ECP and Eotaxin levels in blood of rats (A): represents the morphology of separated EOS under microscope; (B): represents the morphology of EOS after Wright-Giems staining; (C): represents the EOS result in serum; (D): represents the ECP level in BALF; (E): represents the Eotaxin level in BALF. Data are expressed as mean±SEM (n=8). (Control: normal-controlled group; Model: model-controlled group; ECT: ECT treated group; DXMS: positive-controlled group.). Significant differences #P<0.05, ##P<0.01, ###P<0.001, compare with control group; *P<0.05, **P<0.01, ***P<0.001, compare with model group.

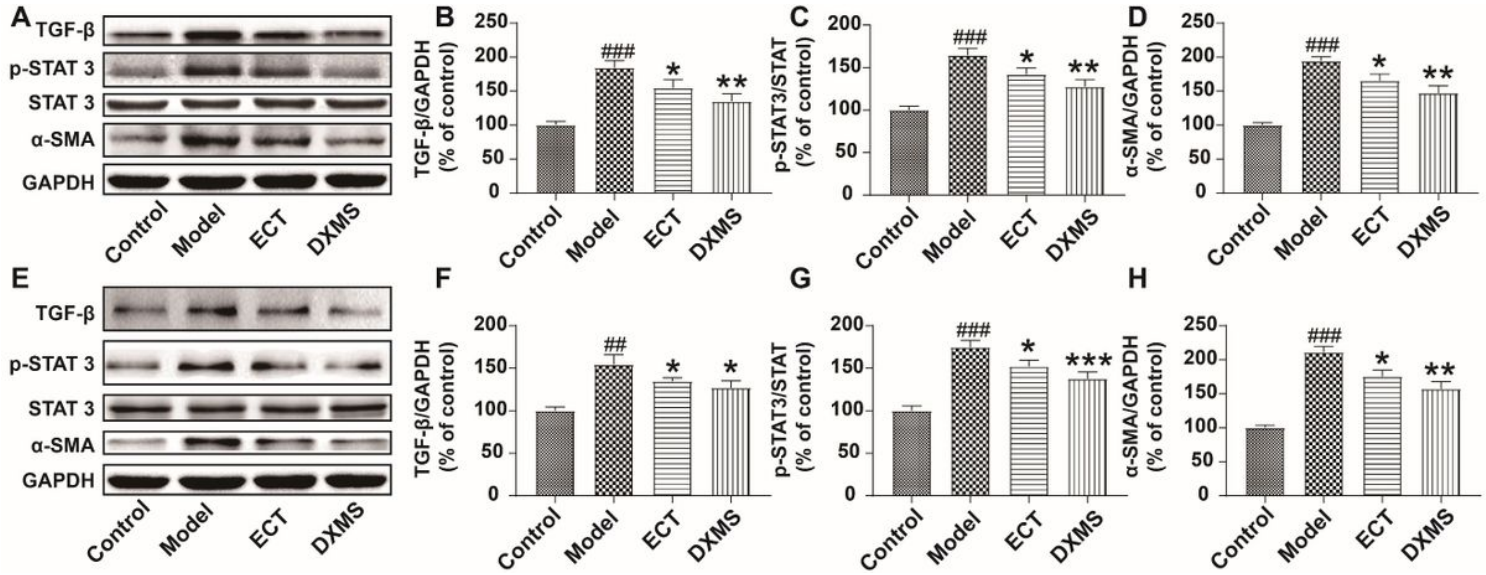

Figure 7

Protein expression of TGF-β/STAT3 pathway (A): represents the protein levels of TGF-β/STAT3 signaling pathway in BALF; (B): represents the levels of TGF-β/GAPDH (% of control) in BALF; (C): represents the levels of p-STAT3/STAT (% of control) in BALF; (D): represents the levels of α-SMA/GAPDH (% of control) in BALF; (E): represents the protein levels of TGF-β/STAT3 signaling pathway in separated EOS cells; (F): represents the levels of TGF-β/GAPHD (% of control) in separated EOS cells; (G): represents the levels of p-STAT3/STAT (% of control) in separated EOS cells; (H): represents the levels of α-SMA/GAPDH (% of control) in separated EOS cells. Data are expressed as mean±SEM (n=8). (Control: normal-controlled group; Model: model-controlled group; ECT: ECT treated group; DXMS: positive-controlled group.). Significant differences #P<0.05, ##P<0.01, ###P<0.001, compare with control group; *P<0.05, **P<0.1, ***P<0.001, compare with model group.