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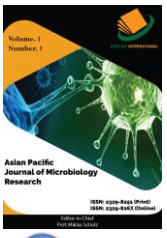

EXPRESSION OF NF-KBMRNA IN ASTHMA RATS LUNG BASED ON THE EFFECT OF DIFFERENT TCM TREATMENT

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ABSTRACT

To detect expression of NF-κB gene in the asthmatic rats lung tissue to explore the effect of NF-κB in asthma rats airway remodeling based on Real-time quantitative PCR. Then to research the effect of different TCM interventions for its role. Asthma rat models were prepared by injecting ovalbumin (OVA) into the abdominal cavity and inhalation of aerosol, and intervented by the different TCM treatment methods, example for body resistance-strengthening, lung-diffusing, lung-diffusing and body resistance-strengening. Then detected expression of NF-κB gene in the asthmatic rats lung tissue based on Real-time quantitative PCR. And compared lung coefficient between the rats in each group. The results showed that the three prescriptions can all reduce the expression of NF-KB in the lung tissue, there is statistical significant difference between model group and lung-diffusing and lung-diffusting and body resistance-strengthening group. The three treatment methods can reduce the lung coefficient. NF-κBmRNA is Gene NF-κB in airway remodeling in asthmatic rats has important value and different TCM treatment is effect to it. This is consistent with TCM theory understanding to asthma. The pathogenesis of asthma is deficiency of healthy Qi and exuberance of evils, then the treatment principles should strengthen healthy Qi to remove toxic substance.

KEYWORDS

Asthma rats, NF-κB gene, Lung coefficient, Traditional Chinese Medicine Treatment.

1. INTRODUCTION

Many Asthma is a kind of polygenic hereditary diseases caused by genetic factor and environmental factor, and showed obvious familial aggregation. Nuclear factor kappaB (NF-κB) is one of the key factors that regulate cellular gene transcription. Activated NF-κB has direct important effect among reaction of organism inflammation and immune, and related closely with occurrence and development of diseases. Traditional Chinese Medicine (TCM) better intervene in many diseases based on regulate the activation of NF-κB. Based on a study, the Chinese Traditional Medicine (referred to as TCM) has a good role in the treatment of bronchial asthma based on the general principle of "overall adjustment, strengthening and restoring the balance of yin and yang". Confirmed by clinical and animal experiments, TCM treatment methods, such as lung-diffusing, resistance-strengthening, lung-diffusing and resistance-strengthening, has lots of effects such as anti-asthmatic, antispasmodic, anti-allergic and immune regulation [1-4]. Relative to western medicine, TCM pay more attention to the overall treatment of the disease. TCM/s characteristic as Multi-channel, multi-link and multi-target showed good prospects in the prevention of airway remodeling [5-7].

The study observed three treatment methods for asthma rats, such as lung-diffusing, resistance-strengthening, lung-diffusing and resistance-strengthening, affect the lung coefficient and the expression NF-κBmRNA. Then to explore the effect and mechanism of different TCM treatment to airway remodel of asthmatic rat.

2. MATERIALS AND METHOD

2.1 Animal grouping

Sixty healthy SD male rats, weight 200±20 gram were randomly divided into six groups: normal control group (group A), model group (group B), prednisone group (group C), body resistance-strengthening group (group D), lung-diffusing therapy group (group E), lung-diffusing and body resistance- strengthening group (group F).

2.2 Modeling method

Asthma rat models were prepared by injecting ovalbumin (OVA) into the abdominal cavity and inhalation of aerosol based on the modeling method described in reference (Wasemmn et al.,1992; Lv et al.,1995). On first day after animal grouping, each rat of group B,C,D,E,F was injected intraperitoneally solution 1mL(contain of OVA 100mg, Aluminum hydroxide 100 mg), after fourteen days, aerosol inhalation 1%OVA 20min, flowing 40ml/20min, continued 28 days. Rats in group A were injected 0.9%NaCl 1ml, aerosol inhalation 0.9%NaCl 40ml/20min, continued 28 days.

2.3 Drug intervention

Dexamethasone tablets (Shanghai, Xinyi pharmaceutical factory Co.,Ltd. No:H3120793-01) were made 0.32mg/ml suspension with 0.9%NaCl. Prescription of body resistance-strengthening is made of Shengdi10g, Shudi10g, Dangshen10g, Fuling9g, Huangjing9g, Yuzhu9g, Xianlingpi9g, and so on. Prescription of lung-diffusing is made of Mahuang4g, Banxia9g, Chaihu9g, Huangqin9g, Tinglizi9g, and so on. Prescription of lung-diffusing and body resistance-strengthening is made of Shengdi10g, Shudihuang10, Dangshen10, Zexie10g, Huangjing10g, Xianlingpi10g, Nanshashen9g, Beishashen9g, Banxia9g, Dilong9g, Huangqin9g, and so on. The three prescription is respectively added water to soak for one hour, the first dose, add water to 10 times the total amount of drugs, second dose add water to 4 times, take two time concoction together, evaporation and concentration to solution of 3g/ml crude drug concentration. The first day after asthma induced, the rats of group A,B are given normal saline by gavage, and the rats of group C are given Dexamethasone by gavage, the

rats of group D, E, F are respectively given Prescription of body resistance-strengthening, Prescription of lung-diffusing, Prescription of lung-diffusing and body resistance-strengthening by gavage., one time every day, dose of 10ml/kg·bw. After four weeks, drawing materials and detecting relevant indicators.

2.4 Detecting indicators and method

Researching the expression of NF-κBmRNA in asthma rats lung based on Real-time quantitative PCR instrument. Lung coefficient.: Rats were killed after the end of treatment, then removed the whole lung and weighed. Lung coefficient=Lung weight(g)/ body weight(kg).

2.5 Statistical Analysis Method

The measurement data of each group is expressed to x ±S, analyzed by the software of SPSS18.0, single-factor analysis of variance, the difference between the groups using the T-test, $P<0.05$ for the difference was statistically significant.

2.5 Reagents

Reagents used in the experiments are showed in Table 1.

Table 1: Reagents used in the experiments

Reagents	Company of Reagent production (or purchase)
Trizol	Invitrogen
DEPC water	Shanghai DoBio Biotech CO.,LTD
dNTP	Shanghai DoBio Biotech CO.,LTD
Taq enzymes	Takara Biotechnology CO.,LTD
Random primer	Invitrogen
RNA enzyme inhibitor	Invitrogen
AMV	Invitrogen
Sybrgreen	Invitrogen
TBE	Shanghai DoBio Biotech CO.,LTD
6*Loading buffer	Shanghai DoBio Biotech CO.,LTD

2.6.1 Instruments

Instruments used in the experiments are showed in Table 2.

Table 2: Instruments used in the experiment

Instruments	Company of Instruments production
Tissue Ruptor	QIAGEN
Desktop high-speed centrifuge PICO 17	Thermo
UV spectrophotometer Unico UV-2000	Unico (Shanghai) Instrument CO.,LTD.
BIO RAD POWER PAC 3000	BIO RAD
BIO RAD DNA SUB CELL	BIO RAD
Gel Imaging System GIS-2008	Tanon Science & Technology CO.,LTD.
ABI STEP ONE PLUS Real Time PCR System	Applied Biosystems CO.,LTD

2.6.2 Total RNA extraction from lung

RNA was extracted in accordance with the following steps: To take the size of soybean piece of lung tissue in a 1.5ml Eppendorf tube no RNA enzymes and no pyrogenic. add into 1ml Trizol, then place the QIAGEN tissue ruptor homogenized and at room temperature for 10 min; Add 20μl Chloroform, Mix well, 15,000 rpm centrifugal 5-7min; Extract supernatant to 1.5 ml eppendorf tube, add 600μl Chloroform, then mix, 15,000 rpm centrifugal 5 min; Extract supernatant to 1.5ml Eppendorf tube, add 500ul Isopropanol, then mix, 15,000 rpm centrifugal 10 min; The supernatant was discarded, wash with 75% ethanol in 1ml, 15,000 rpm centrifugation 5min; The supernatant was discarded, RNA pellet was dried in air(2-3min); After drying, the RNA was dissolved in DEPC treated water; The total RNA was appropriately diluted, the OD260/OD280 ratio was measurement by spectrophotometric, and quantitatively. Quantitative formula: RNA concentration(μg/ml)=data of OD260×40×Dilution factor (in this experiment was 400-fold).

2.6.3 Reverse transcriptase

Adding the following reactants in a 1.5ml centrifuge tube by the following sequence (volume of 12.5ul): DEPC water(8-x)μl, 50U/ul RNA Enzyme Inhibitors 0.5μl, 50pM/ul Random primer 2μl; RNA xμl(2ug), the total volume of the total RNA with DEPC water 8μl, where in the amount of RNA is 2μg; In a water bath at 65℃ for 5min; At room temperature for 10min, 5000rpm centrifugation 5 seconds; Adding the following reactants to the 1.5ml centrifuge tube by the following sequence (added after a total volume of 20ul) 50U/ul RNA Enzyme Inhibitors 0.5μl, 5×buffer4μl, dNTP MIX(10mM/each) 2μl, DTT 2μl, AMV(200U/ul) 1μl; Water bath at 40℃ reaction for 1 hour; Handle 5-10min at 90℃; Ice bath 5min; High-speed (above 5000rpm) centrifuged for 5 seconds.

2.6.4 Quantitative PCR amplification

(1) Design of sequence and primer

Sequence and primer are devised based on Primer Express2.0 software in Invitrogen Co.,Ltd. These sequence and primers are showed in table3.

Table 3: The sequence and primers used in the experiment

5	Primers	Probe	Annealing temperature
GAPDH	751F 5-GCCAAGTATGATGACATCAAGAAGGT-3 819R 5-GCCCAGGATGCCCTTTAGTG-3	779T 5-FAM-TGAAGCAGGCGGCCGAGGGC-TAMARA-3	60℃
NF-KB	44F 5-AGGCTTCTGGGCCATATGTG-3 156R 5-TGTGCTTCTCTCCCCAGGAA-3	110T 5-FAM-AGTGCGAGGGCCGCTCTGCA-TAMRA-3'	60℃

(2) PCR reaction system

The PCR reaction system carried on by the following condition. Showed in Table 4.

Table 4: PCR reaction system（25μl）

reaction	Volume
H_2O	15.1μl
$MgCl_2$ (TaKaRa)	2μl
Sybr green	0.5μl
10×buffer (TaKaRa)	2.5μl
d NTP (10mM/each)	2μl
Primer (up50pM/ul)	0.3μl
Primer (down50pM/ul)	0.3μl
Template (cDNA)	2μl
Taq (5U/ul)	0.3μl
Total	25μl

(3) Reaction conditions

The reaction conditions are showed in Table 5.

Table 5: Reaction condition

Temperature	Time	cycles
95°C	2min	
94°C	10s	40cycles
60°C	10s	40cycles
72°C	40s	40cycles

3. RESULTS

Comparison of $2^{-\Delta\Delta CT}$ value AcDNA template that was sure to be given rise to the gene to be examined was chosen to be a primer and the reaction solution was prepared. Real-time quantitative PCR was performed with the primer express2.0 from Invitorgen Company. The $2^{-\Delta\Delta CT}$ method is a convenient way to analyze the relative changes in gene expression from real-time quantitative PCR experiments. Data analysis of this report used the $2^{-\Delta\Delta CT}$ method (Kenneth et al.,2001; Michael Wet al.,2002).The result of $2^{-\Delta\Delta CT}$ value is showed intable6.

Table 6: The comparison of $2^{-\Delta\Delta ct}$ in groups

Groups	$2^{-\Delta\Delta CT}$ number ($\bar{x}\pm S$)
Group A	0.6676±0.2279▲
Group B	1.5496±1.0829★
Group C	0.8916±0.5833
Group D	1.2607±0.8577
Group E	1.0385±0.4897
Group F	0.6798±0.1223▲

Note: compared with group A, ★ P <0.05; compared with group B, ▲ P <0.05

Showed from table6, Compared with group A, the $2^{-\Delta\Delta CT}$ in the lung of rats in group B increased, there isstatistical significant difference between group A and group B. Compared with group B, the $2^{-\Delta\Delta CT}$ in the lung of rats in group F reduced, there is statistical significant difference between group C and group F.

3.1 Comparison of lung coefficient

The compare of lung coefficient between the rats in each group is showed in Table 7.

Table 7: Comparison of lung coefficient

group	n	Lung coefficient ($\bar{x}\pm S$)
Group A	10	7.3306±1.1359▲
Group B	9	9.1493±1.8564★
Group C	9	10.4569±1.5721★
Group D	9	8.3216±1.1054▲
Group E	10	8.5209±1.7753▲
Group F	10	8.1388±1.8997▲

Note: compared with group A, ★ P <0.05; compared with group C, ▲ P <0.05

Showed from table7, Compared with group A, the lung coefficient of rats in group B, C increased, there is statistical significant difference between group A and group B,C; Compared with group C, the lung coefficient of rats in group A, D, E, F reduced, there is statistical significant difference between group B and group A, D, E, F.

4. DISCUSSION

Research data shows NF-κB is the major nuclear transcription factor of regulation of inflammatory and immune responses and plays a critical role in signal transduction on cell immunity, infection and apoptosis [8-12]. NF-κ B not only related with B cell differentiation and function, but also T cell, thymus cells, macrophages, fibroblasts and neuronal differentiation and function. Therefore, NF-κB is not only involved in pathological processes of infection, inflammation, stress, immune response, apoptosis and cancer, but also involved in cell cycle and the control of cell differentiation, and so on. Under normal conditions, NF-κB expression is low, increased expression in inflammation occurs, in order to maintain the stability of the structure of the body and participate in the body's immune response.. When over expressed, it would lead to increase inflammatory protein, then lead to disease. Studies have shown that the activation of NF-κB pathway can achieve the purpose to improve the immune system, activation of the NF-κB pathway with each other to promote the role of the immune system [13-15]. The Chinese medicine can play a good intervention to the clinical treatment of a variety of diseases through the regulation of NF-κB activation [16-19]. The visceral organ weight and organ coefficient (organ weight / body weight ×100%) of experimental animals is one of the main biological characteristics, and it is an important basis for the identification of animal genetic equality. Among the biomedical research, it can also be used to measure and reflect the functional status of animals [20].The organ coefficient changes often can better reflect the chemical poisons to this organ toxicity, and it can evidence the possibility of circumstantial histopathological changes, it is also an important clue to find the target organ of the poison role [21]. In this study, we found Dexamethasone and the TCM treatment can all reduce the lung coefficient of rats, but the TCM treatment is better than Dexamethasone.

Ancient physicians believe that the phlegm volts lung is the radical reason of asthma. Phlegm is cited to touch while suffering external factors. Phlegm raises by following Qi, Qi is blocked because of phlegm, they kink each other, then airway is blocked and lung-Qi rises and drop disorderly [22]. That cause phlegm as a roar and the breath of breathlessness. Lung, spleen and kidney are role during asthma remission, but there is still the airway inflammation and airway hyper responsiveness still airway obstruction phenomenon. Clinical studies have shown that prescription of body resistance-strengthening could invigorate lung spleen and kidney, and nourish Qi, blood and Yin, Yang. It focuses on regulating the body/s immune resistance to disease. Prescription of lung-diffusing can ventilate lung to relieve dyspnea, dissolve phlegm and stop cough, clear lung and antispasmodic, relieve asthma attacks, it is effective exacerbation of treating asthma [23]. Prescription of lung-diffusing and body resistance-strengthening can take into account the supplementation and attack. The rat model of asthma copied by OVA is similar to asthma state, but it needs to be confirmed which syndrome it belongs to TCM theory. This study showed the three prescriptions can all reduce the expression of NF-KB in the lung tissue, there is statistical significant difference between model group and lung-diffusing and body resistance-strengthening group. This is consistent with TCM theory understanding to asthma. The pathogenesis of asthma is deficiency of healthy Qi and exuberance of evils, then the treatment principles should strengthen healthy Qi to remove toxic substance.

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