ISSN : 0974 - 7435 Volume 10 Issue 21

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(21), 2014 [13508-13512]

Effect of compound-maqin-decoction on expression of th17/treg cytokines in asthmatic rats

Xie Yan-hong, Li Xue-liang, Xu Zhao-xia*, Qian Peng, Li Xue-ping, Xu Jin
Shanghai University of Traditional Chinese Medcicine, Shanghai, (CHINA)
E-mail: zhaoxia7001@126.com

ABSTRACT

In the current study, asthma rats model replicated, and interventionwith CMD.After 4 weeks of treatment, detect the serum expression of TNF-α and IL-10 in rats, IL-22 and IL-35 in lung homogenate. Compared with the normal group, the model group, dexamethasone group and small doses of TCM group rats serum TNF-αrise and IL-10 reduce, the difference was statistically significant, and no significant differences between with large doses of TCM group. After treatment, the serum TNF-αreduce and IL-10 rise, the difference between large doses of TCM group, model group and dexamethasone group was statistically significant. Compared with the normal group, the model group, each group tissue homogenate IL-22 rise and IL-35 reduce, the difference was statistical significant, and no significant differences between with large doses of TCM group. After treatment, the lung homogenate IL-22 reduce and IL-35 rise, the difference between large doses of TCM group, model group and dexamethasone group was statistically significant. These results showed that CMD maybe inhibit the generation of TNF-α, IL-35, and promote the body to release inflammatory cytokines like IL-10, IL-22, etc, to adjust the balance of Th17/Treg cells, reduce rats airway inflammatory reaction and relieve asthma symptoms.

KEYWORDS

Asthma; Compound-maqin-decoction (CMD); TNF-α; IL-10; IL-22; IL-35.

INTRODUCTION

Asthma pathogenesis is very complex. Chronic airway inflammation and hyperresponsiveness is considered as the main feature, and there are many factors involved. Helper T cells (Th) is a new effect T cell subsets found in recent years, that cells have an independent regulatory mechanisms of differentiation, and play an important role in the defense of the extracellular pathogen infection, autoimmune diseases and infectious diseases[1]. Regulatory T cells (Treg) is a T-lymphocyte subsets that has immunosuppressive function. The imbalance of Th17/Treg cells is an important factor of infectious diseases[2]. Compound-Maqin-Decoction (CMD) can regulate immune function, inhibit inflammation, relax bronchial smooth muscle, control airway inflammation, and improve lung function in asthmatic guinea pigs. These findings have been confirmed by clinical and experimental studies in the past 20 years[3-6]. In the current study, we collected the following data to determine the effect of CMD on airway inflammation in asthmatic rats: serum tumor necrosis factor (TNF)-α and interleukin (IL)-10 expression, and lung homogenate IL-22, IL-35 expression. We expect to investigate the asthma pathogenesis and the effector mechanisms of CMD.

MATERIAL AND METHODS

Animal grouping

Fifty healthy SD male rats (Shanghai SLAC Laboratory Animal Co., Ltd, Shanghai, China), weighing 200 ± 20 g, were randomly divided into five groups, as follows: normal control group (Group A); model group (Group B); dexamethasone group (Group C); large doses CMD group (Group D) and small doses CMD group (Group E), 10 each.

Modeling method

The asthma rat model was prepared by injecting ovalbumin (OVA) into the abdominal cavity and inhalation of aerosol based on the modeling method previously described[7-8]. On the first day after animal grouping, each rat in groups B, C, and D was injected intraperitoneallywith 1 ml of solution containing 100 mg of OVA and 100 mg of aluminum hydroxide.After 14 days, an aerosol inhalation of 1% OVA was administered for 20 min ata flow rate of 2 ml·min−1, and continued for 28 days. The rats in group A were injected with 1 ml of 0.9% sodium chloride solution and an aerosol inhalation of 0.9% sodium chloride solutionwas administered a flow rate of 2 ml·min−1, and continued for 28 days.

Treatment intervention

Dexamethasone tablets (No. H3120793-01; Shanghai XinyiPharmaceutical Factory Co.,Ltd., Shanghai, China) were prepared as a suspension (0.32mg·mL−1)with 0.9% sodium chloride solution.CMD consists of Zhimahuang (Ephedra; 4 grams), Huangqin (Scutelleria; 9grams), Cangerzi (Xanthium; 9grams), Tianzhuzi (Nandinadomestica; 9 grams), Lameihua (Chimonanthus praecox flowers; 9grams), and Hutuiziye (Elaeagnus leaf; 9 grams). These herbs of the prescriptions were soaked in water for 1 h.Tenvolumes of water were added during the first time cooking. Four volumes of water were added during the second time cooking. Then, the prepared decoctions from both cooking times were collected, evaporated, and concentrated into a3g·mL−1 crude drug solution.

The first day after asthma was induced, rats in group A and B were given normal saline by gavage, rats in group C were given dexamethasone by gavage, rats in group D and E wererespectivelygiven large and small doses CMD decoction by gavage (10 ml·kg−1 body weight once daily). After 4 weeks, materials were collected for the detection of relevant indicators.

Detection method and indicators

Serum prepared: Serum preparation was to use test tube free ofpyrogen and endotoxin. And other cell stimulations should also be avoided during the operation. The blood was collected and centrifuged for 10 minsat 3000 rpm. Then, the supernatant was collected and kept at -20°C.

Lung homogenates prepared: The lung tissue blocks weighing 0.2g ~ 1g, rinsed in ice-cold saline to remove blood, paper drying, weighed, taken into a small beaker of the 5 or 10ml.Take cold 0.86%saline, the total volume of saline is nine times the weight of the lung tissue blocks, using a pipette to take two-thirds of the total amount of saline in a beaker, crushed quickly tissue blocks with a small scissors ophthalmic (operation to be carried out in an ice bath).The lung tissue was imported into the glass homogenizer tube, then flush the crushed tissue blocks in the beaker with remaining 1/3 saline, left together into homogenizer tube to homogenize, fully crush, the lung tissue homogenization.The lung homogenates prepared centrifuged for 10 minsat 3000 rpm. Then, the supernatant was collected to reserve.

The serum expression of TNF-αand IL-6, and the expression lung homogenates of IL-22, IL-35 was tested by ELISA. ELISA kit purchased from Shanghai ShiruiBiotechnology Co., Ltd (TNF-α No: CK-E30635R; IL-10 No:CK-E93207R; IL-22 No:CK-E93201R; IL-35 No:CK-E93202R).

Steps were carried out strictly in accordance with instructions included in the ELISA kit. To increase the accuracy of data, within 15 mins of adding the stop solution, the wavelength (OD) value was measured in a microplate reader at 450 nm.

The OD value was recorded every 3 mins for a total of four times. The average value of 4 OD readings was achieved. The standard curve (R2 values ≥0.99) was obtained using SPSS software.

Statistically analysis

All the experimental data were analyzed statistically by SPSS18.0 statistical software. ANOVA was used for the analysis of difference betweengroups. The inspection level was $\alpha = 0.05$. And the $P < 0.05$ was considered as statistically significant difference.

RESULT AND DISSCUSS

General observation

The rats in groups A are good, lively and good move, supple fur, weight gradually increased, breathing smoothly and evenly. The rats in group B are worse than group A, apathetic, slow, gloss hair down, after induced asthma, the rats in group B areirritability, shortness of breath, sneezing or cough, mild cyanosis, muscle twitching, unresponsive and other symptoms. Afterthe treatment by Dexamethasone and CMD, above asthma symptoms relieve, and the symptoms of large dose CMD group is better than two treatment groups.

Expression of lung tissue morphology in the rats

Lung tissue of the rats in group A has no significant damage, the structure is normal, bronchial epithelial is integrity, no exudate in the lumen, no significant inflammatory cell infiltration. The rats in group B, bronchial epithelial cell damage and loss, the epithelial cells can be seen in cavity shedding, smooth muscle fibers mild hyperplasia, pulmonary arterial wall thickening. The rats in group C, bronchial epithelial slightlyedema, much exudate in cavity, there arefew inflammatory cells infiltration on the tube wall. The rats in group D, a few inflammatory exudatecan be seen in endobronchial. The rats in group E, bronchial epithelial shedding a small amount, the lumen filled with exudate, inflammatory cell infiltration in perivascular.

Expression of TNF-α and IL-10 in serum of rats

Compared with group A, the expression of TNF-α in serum of rats in group B and C was increased, and IL-10was decreased, there werestatisticallysignificant differences; there were no statisticalsignificant differences between group A and D. After treatment, the expression of TNF-α in serum of rats was decreased and IL-10 was increased, there werestatisticalsignificant differences between group D and B, C, but there were no statisticalsignificant differences between group E and B, C. The results are shown in TABLE 1

TABLE 1: Comparison of serum expression of TNF-α and IL-10 in rats ($\bar{x}$ ±S)

Groups	TNF-α (ng/ml)	IL-10 (ng/ml)
GroupA (n=10)	0.0544±0.0544	0.0552±0.0129
GroupB (n=10)	0.0893±0.0210★	0.0393±0.0132★
GroupC (n=10)	0.0856±0.0356★	0.0422±0.0127★
GroupD (n=10)	0.0485±0.0098■▲	0.0521±0.0119■
GroupE (n=10)	0.0799±0.0212★	0.0467±0.0180

Note: Compared with group A, ★*P<0.05*; Compared with groupB, ■*P<0.05*; Compared with group C,▲*P<0.05*

Expression of IL-22 and IL-35 in lung homogenates of rats

Compared with group A, the expression of IL-22 in lung homogenates of rats in group B, C and Ewas increased, and IL-35was decreased, there werestatisticalsignificant differences; there were no statisticalsignificant differences between group A and D. After treatment, the expression of IL-22 in serum of rats was decreased and IL-35 was increased, there werestatisticalsignificant differences between group D and B, C, but there were no statisticalsignificant differences between group E and B, C. The results are shown in TABLE 2.

TABLE 2 : Comparison of lung homogenates expression of IL-22 and IL-35 in rats ($\bar{x}$ ±S)

Groups	IL-22 (ng/ml)	IL-35 (ng/ml)
GroupA (n=10)	0.0011±0.0003	0.0128±0.0011
GroupB (n=10)	0.0015±0.0005★	0.0104±0.0011★
GroupC (n=10)	0.0015±0.0004★	0.0105±0.0013★
GroupD (n=10)	0.0011±0.0004■▲	0.0124±0.0010■▲
GroupE (n=10)	0.0016±0.0004★	0.0121±0.0007■★

Note: Compared with group A, ★*P<0.05*; Compared with groupB, ■*P<0.05*; Compared with group C,▲*P<0.05*

DISCUSSION

The biological functions and differentiation of Th17 and Treg is antagonistic, proinflammatory Th17 cells and inflammation Treg cells keep in balance by restrict each other. In recent years, the imbalance of Th17/Treg cells in the pathogenesis of asthma is increasingly attention. IL-10 is a inflammatory cytokines which secreted by Treg cell, it can play the role of inhibition of T cells and eosinophils, to inhibit of the generate proinflammatory cytokines and chemokines[9]. IL-10 can prevent T cell receptor mediated excitation of CD4+T cells, T cells are processed with IL-10 can lead to lasting antigen specific T cells without effect[10], to achieve its anti-inflammatory effects. TNF-αis produced by mononuclear macrophages, peptide regulatory factor with a broad range of biological activities. Its biological activity performance is according to the different concentration[10]. It has low concentration in the body, with a variety of physiological functions such as in regulation of the immune response and anti-infection, and possesses protective effects on the body. But the high concentration of TNF-αis an important inflammatory mediator, it can mediate many pathophysiological process of inflammatory response, and cause local inflammation response. IL-22 is secreted by the Th17 cells and other cells, can participate in antimicrobial defense, protect and repair tissue injury and acute phase reaction[11]. When inflammation or infection, it can be as the first line in the host defense system, in order to under the condition of Th1 type immunodeficiency, to maintain the integrity of the epiderma[12]. IL-35 is a kind of cytokine which was recently found, it is closely related to the immunoregulatory effects, such as imbalance of Th17/Treg cells. It can through various channels to participate in the pathogenesis of asthma, become the new target for this phase asthma treatment. IL-35 can effectively improve Treg subsets play an inhibitory effect, to inhibition of Th17 cell proliferation differentiation, and restrain excessive immune response and immunologic injury[13].

Compound-Maqin-Decoction (CMD) consists ofEphedra 4 grams, Scutelleria 9 grams, Xanthium 9 grams,Nandinadomestica 9 grams, Chimonanthus praecox flowers 9 grams, Elaeagnus leaf 9 grams and other medicinal materials. Preliminary studies confirmed that this prescription can obviously relieve the clinical symptoms of asthma, and improve lung function. Our study shows: (1)CMD can improve the asthmatic rats' identification, such as diet, fur color and luster,defecation and so on. Effectively relieve the inflammatory infiltrates of bronchial tissue. (2) For the expression of cells cytokines in serum of asthma rats, compared with the normal group, after excitation caused asthma, each treatment groups serum TNF-α levels risesignificantly. TNF-αas a kind of earlyinflammatory mediator, it can cause the damage of thelung tissue, which interfere with the body's immune regulation mechanism, resulting in increased illness. However, the IL-10 expression in the other three treatment groups were reduced,indicate the patients with bronchial asthma, whose secretion of IL-10 is reduce. Cannot effectively suppress the inflammatory response, may be one of the reasons lead to or aggravate the airway inflammation. Compared with model group, large doses of CMD groupcan reduce the content of serum TNF-α and increase the content of IL-10 at the same time.And compared with the dexamethasone treatment group, the adjustment range is greater and the curative effect is more significant. (3)For the expression of cells cytokines in lung tissue homogenate of asthma rats, compared with the normal group, after excitation caused asthma, each treatment groups serum IL-22 levels rise. Show that play an important role in participation in the airway reconstruction and promote the airway inflammation more seriously. The IL-35 expression was reduced, and after treatment the three groups' IL-35 express were increased accordingly. Indicates that it can plays an immune regulation and relevant treatment function in bronchial asthma and other allergic diseases. Compared with model group, large doses of CMD groupcan increase the content of serum IL-22 and reduce the content of IL-35 at the same time, and the effect is superior to the use of glucocorticoid. Therefore, this study suggests, CMD on the one hand can inhibit the generation of TNF-α, IL-35 reduce rats airway inflammatory reaction and promote tissue repair,promote the body to release inflammatory cytokines like IL-10, IL-22, etc, to adjust the balance of Th17/Treg cells, So as to relieve airway inflammation and airway reconstruction, improve asthma symptoms.

ACKNOWLEDGMENTS

This study was supported by the Shanghai Natural Science Foundation (No.14ZR1441500) and the Shanghai TCM Special Funds Project of Health and Family Planning (No. 2014JP029A).

REFERENCES

[1] An Xia, Gong Ying, Ye Ling, et al.; Advance on studies of T helper 17 cells and its relative cytokines and bronchial asthma, Chinese Journal of Asthma, **6(1),** 26-29 **(2012)**.

[2] Zhang Wei, Wang Xinwei, Liu Xinnian; Clinical significance of combined detection of Th17 and Treg cells in 68 cases of bronchial asthma, Chongqing medical journal, **42(14)**, 1583-1585 **(2013)**.

[3] Zhu Huihua, Yu Jianer, Zhang Xiaofeng, et al.; Effects of Pingchuan Recipe on airway remodeling in mice with bronchial asthma, Journal of Integrative Medicine, **03**, 261-267 **(2009)**.

[4] Wang Yiqin, Li Fufengi, TengZhongzhao; Research of the airway reconstruction characteristics in bronchial asthmatic rats, Chinese Journal of Tuberculosis and Respiratory Diseases, **26(11)**, 728-729 **(2003)**.

[5] Wang Yiqin, Li Fufeng, Yan Haixia, et al.; Combination Ephedra and Scutellaria Decoction Regulates Immune Function of Child with Asthma, Shanghai Journal of Tradition Chinese Medicine, **40(4)**, 37-38 **(2006)**.

[6] TengZhongzhao, Wang Yiqin, Li Fufeng, et al.; Tracheal compliance and limit flow rate changes in a murine model of asthma.Science in China Series C, **51(10)**, 922-931 **(2008)**.
[7] S.Wasemmn, R.Olivenstein, P.M.Renzi; The Relationship between Late Asthma tic Responses and Antigen-Specific Immunoglobulin, Allergy ClinImmunol, **90**, 661-669 **(1992)**.
[8] QiaoHongmei, PangHuanxiang, ZhangYunfeng, et al.; Changes of IL-6, IL-10 and TNF-α in children with Mycoplasma pneumoniae pneumonia, Journal of Chinese Pediatrics, **1**, 59-61 **(2012)**.
[9] Shao Li, GuoYinshi, ZhuLijun, et al.; Changes of serum IL-10, IL-5, and ECP levels in bronchial asthmatics and the clinical significance, Immunological Journal, **1**,76-78 **(2008)**.
[10] I.Bellinghausen, J.Knop, J.Saloga, et al.; The role of interleukin-10 in the regulation of allergic immune responses, Int Arch Allergy Immunol, **126(2)**, 97-101 **(2011)**.
[11] Sun Qi; IL-22: the key cytokine in inflammatory diseases, Immunological Journal, **09**, 821-825 **(2011)**.
[12] A.De Luca, T.Zelante, C.D'Angelo, et al.; IL-22 defines a novel immune pathway of antifungal resistance, Mucosal Immunol, **3(4)**, 361-373 **(2013)**.
[13] Gu Xian; Study of the immunomodulatory and therapeutic effects of recombinant Interleukin-35 on asthma in mice, The second Military Medical University, **(2013)**.