

Effects of *Wuwei Dilong* Decoction on Inflammatory Cells and Cytokines in Asthma Model Guinea Pigs

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Objective: To explore the effects and the mechanism of *Wuwei Dilong* Decoction (五味地龙汤 *Schisandra Fruit and Earthworm* Decoction) for treatment of asthma. **Methods:** The asthma guinea pig model was established with spray of ovalbumin (OVA). Fifteen days later, the guinea pigs were administered by intra-gastric perfusion of *Wuwei Dilong* Decoction once a day for 8 consecutive days. Blood samples were taken for testing the total leucocytes, eosinophil (EOS), lymphocytes, interferon- γ (IFN- γ) and leukotriene B₄ (LTB₄). **Results:** In the asthma model group, the total leucocytes, EOS and lymphocytes were all increased, with significant differences as compared with the different dosage *Wuwei Dilong* Decoction groups ($P < 0.01$ or $P < 0.05$). The serum LTB₄ in the asthma model group was significantly increased and IFN- γ decreased. After administration of *Wuwei Dilong* Decoction of the large, medium and small dosages, LTB₄ decreased, while IFN- γ increased ($P < 0.05$ or $P < 0.01$). **Conclusion:** *Wuwei Dilong* Decoction can inhibit infiltration and diffusion of the inflammatory cells in the asthma model guinea pigs, and regulate LTB₄ and IFN- γ , which is probably one of the important mechanisms of *Wuwei Dilong* Decoction for relieving asthma.

Key words: asthma; cytokines; TCM therapy; *Wuwei Dilong* Decoction

Bronchial asthma is caused by chronic inflammation of the respiratory tract in which many inflammatory cells, such as eosinophil (EOS), mast cells, T-lymphocytes, etc. are involved. In order to probe into the mechanism of *Wuwei Dilong* Decoction (五味地龙汤 *Schisandra Fruit and Earthworm* Decoction), the effects on the total leucocytes, eosinophil (EOS) and lymphocyte counts, interferon- γ (IFN- γ) and leukotriene B₄ (LTB₄) were investigated. A report follows.

MATERIALS

Laboratory Animals

Guinea pigs, aged 2 months, weighing 280 $\pm$ 20g, and equal number of males and females, were supplied by the Center of Laboratory Animals, Tongji Medical College, Huazhong University of Science and Technology, production conformity certificate No:

SCXK (鄂) 2004-0007, laboratory animal conformity certificate No: SYXK (鄂) 2004-0028. And the standard granules forage for laboratory animals were also supplied by the Center of Laboratory Animals, Tongji Medical College, Huazhong University of Science and Technology.

Preparation of *Wuwei Dilong* Decoction

Wuwei Dilong Decoction (*Schisandra Fruit and Earthworm* Decoction), composed of Di Long (地龙 *Pheretima Aspergillum*), Yu Xing Cao (鱼腥草 *Herba Houttuyniae Cordatae*), Chai Hu (柴胡 *Radix Bupleuri*), She Gan (射干 *Rhizoma Belamcandae*), Ting Li Zi (葶苈子 *Semen Lepidii*), Zhi Ma Huang (炙麻黄 prepared *Herba Ephedrae*), Feng Fang (蜂房 *Nidus Vespa*), Wu Wei Zi (五味子 *Fructus Schisandrae*) and Gan Cao (甘草 *Radix Glycyrrhizae*), was supplied by the Pharmaceutical Workshop for

Chinese Medicine, Jingzhou Municipal TCM Hospital. The drugs were immersed in 3-fold water for 30 minutes and decocted for 60 minutes, followed by filtration. The first filtrate was kept in another container; the dregs of the decoction was added with 2-fold water, decocted again for 30 minutes and filtrated. The two filtrates were then put together in a water bath to be concentrated to 1.5 g crude drugs/1ml, and stored in a refrigerator at 4°C for use.

Drugs and Reagents

Dexamethasone (DXM) was produced by Zhejiang Xianlu Pharmaceutical Co. Ltd, batch number: 051263. Ovalbumin (OVA) was made by Sigma Company and packed by Wuhan Life Technique Co. Ltd, batch number: 20041008. Pentobarbital sodium was made by Shanghai Chemical Reagent Company, China Medicinal Group, batch number: F20030816. Aluminum hydroxide was produced by Tianjin Institute of Guangfu Refined Chemical Industry, batch number: 20041229. Guinea pig IFN- γ ELISA kit (Rapid-Bio Lab California, batch number: 03260502) and Guinea pig LTB4 ELISA kit (Rapid Bio Lab, California, batch number: 02040605) were purchased from Shanghai Biological Reagent Company.

Instruments

JSC-OK double-head multi-functional ultrasonic nebulizer, Anshan City Electrical Medical Instrument Factory, China; VEX380 -80 °C low-temperature refrigerator, SANYO Company, Japan; Thermo-Labsystems Multiskan MK3 enzyme labeling analyzer (Fenland); LD4-2A Centrifuge (Beijing); Leica DMLB2 photo-electric microscopic camera (Germeny).

METHODS

Grouping of the Animals

60 guinea pigs selected for the study were randomly divided into 6 groups, i.e. normal control group, asthma model group, DXM (positive medicine) group, *Wuwei Dilong* Decoction large dose, middle dose and small dose groups, 10 guinea pigs in each group.

Modeling and Administration

The asthma animal model was prepared in reference to the literature.¹ Fifteen days after asthma induced by spraying, the medicines were administered by stomach perfusion once a day for 8 days. No medicine was given to the guinea pigs in the model group; DXM 0.001g/kg was administered to the DXM (positive medicine) group; *Wuwei Dilong* Decoction 18 g/kg, 12 g/kg and 6 g/kg were given respectively to the large dose, middle dose and small dose groups; and the normal control group received the same volume of saline.

Sampling

One hour after the last administration, the guinea pigs were anesthetized with intraperitoneal injection of pentobarbital 0.03 g/kg, and the carotid artery was immediately separated to take 8ml blood. One part of the blood was used for counts of total blood white cells, EOS and lymphocytes, and another part was still placed for 2 hours and then centrifuged for 10 minutes at 3000 rpm. The supernatant was taken and stored in a low-temperature refrigerator for use.

Determination of LTB4 and IFN- γ

Determination of LTB4 and IFN- γ were carried out according to the directions of the kits, by using the ThermoLabsystems Multiskan MK3 enzyme labeling analyzer and with the ELISA method.

Statistical method

SPSS10.0 statistical software was used for processing the data, and the data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). One way ANOVA was adopted for multiple comparisons among sample means of the measurement data.

RESULTS

Effects of *Wuwei Dilong* Decoction on Differential Blood Cell Counts in the Asthma Model Guinea Pigs

As shown in Table 1, in the guinea pigs of the normal control group, the total count of white cells, and EOS and lymphocyte counts in the blood were within the normal ranges; in the asthma model group they were all significantly higher than those in the normal

control group (all $P<0.01$); and there were no significant differences among the positive medicine group, the large dose, middle dose and small dose *Wuwei Dilong* Decoction groups as compared with

the normal control group in the total count of white cells, EOS and lymphocyte counts, but with significant differences as compared with the asthma model group ($P<0.05$ or $P<0.01$)

Table 1. Comparison of Differential Blood Cells Counts Among the Groups ($\bar{x} \pm s$, $n=10$)

Group	Dose (g/kg)	Total count of white cells ($\times 10^9/\text{mm}^3$)	EOS (%)	Lymphocytes (%)
Normal	—	6.98 $\pm$ 0.46**	1.17 $\pm$ 0.41**	3.60 $\pm$ 0.88**
Model	—	23.71 $\pm$ 4.03	16.71 $\pm$ 1.11	11.17 $\pm$ 1.06
Positive	0.001	7.53 $\pm$ 0.82**	1.50 $\pm$ 1.00**	5.38 $\pm$ 1.49**
Large dose	18	7.60 $\pm$ 0.52**	1.37 $\pm$ 0.81**	5.43 $\pm$ 0.55**
Middle dose	12	9.30 $\pm$ 0.82**	2.44 $\pm$ 0.53**	7.56 $\pm$ 0.63**
Small dose	6	16.10 $\pm$ 2.30**	5.71 $\pm$ 2.23**	9.39 $\pm$ 0.22*

Notes: Compared with the model group, * $P<0.05$, ** $P<0.01$.

Effects of *Wuwei Dilong* Decoction on the Serum LTB4 and IFN- γ Levels in Asthma Model Guinea Pigs

Table 2 showed that the serum LTB4 content in the asthma model group significantly increased as compared with those in the positive medicine group, the large dose, middle dose and small dose *Wuwei Dilong* Decoction groups, and the normal group (all

$P<0.01$); the serum IFN- γ level in the asthma model group significantly decreased as compared with those in the positive medicine group, the large dose, middle dose and small dose *Wuwei Dilong* Decoction groups, and the normal group ($P<0.01$ or $P<0.05$); there were no significant differences in LTB4 and IFN- γ levels among the other groups except the asthma model group (all $P>0.05$).

Table 2. Comparison of Serum LTB4 and IFN- γ Levels Among the Groups (pg/ml, $\bar{x} \pm s$, $n=10$)

Group	Dose (g/kg)	LTB4	IFN- γ
Normal	—	11.90 $\pm$ 0.51**	35.41 $\pm$ 2.31**
Model	—	17.49 $\pm$ 1.61	18.74 $\pm$ 2.22
Positive	0.001	11.17 $\pm$ 0.68**	39.08 $\pm$ 1.06**
Large dose	18	11.33 $\pm$ 0.85**	35.06 $\pm$ 2.83**
Middle dose	12	11.91 $\pm$ 1.47**	31.80 $\pm$ 1.83**
Small dose	6	12.09 $\pm$ 1.39**	29.74 $\pm$ 1.84*

Notes: Compared with the asthma model group, * $P<0.05$ and ** $P<0.01$.

DISCUSSION

Purging the heat accumulated in the lungs, and relieving asthma and spasms are the important principles of TCM for treatment of asthma. In the prescription of *Wuwei Dilong* Decoction, Di Long (地龙 *Pheretima Aspergillum*) functions to relieve spasms and asthma; Ma Huang (麻黄 *Herba Ephedrae*) can promote the dispersing function of the lungs and relieving asthma; Ting Li Zi (葶苈子 *Semen Lepidii*) can purge the heat accumulated in the

lungs and relieve asthma; Yu Xing Cao (鱼腥草 *Herba Houttuyniae Cordatae*) can clear away phlegm and remove fluid retention from the interior; Chai Hu (柴胡 *Radix Bupleuri*) can regulate functional activities of *qi*; She Gan (射干 *Rhizoma Belamcandae*) can resolve phlegm and relieve sore-throat; Wu Wei Zi (五味子 *Fructus Schisandrae*) can arrest persistent cough and nourish the kidneys, Feng Fang (蜂房 *Nidus Vespae*) can expel pathogenic wind.

In the case of bronchial asthma, EOS, mass cells and T-lymphocytes, etc. are considered to be the main inflammatory cells for formation of chronic air-way inflammation, and they are the central link of the disease. Since the early 20th century, the link between EOS and reversible asthma air-way obstruction has been recognized; and it is a key cell of asthma inflammation and plays the role though releasing the inflammatory medium produced and stored in cells, thus injuring epithelium of the air-way.^{2,3} In the previous studies, the authors found that *Wuwei Dilong* Decoction may show a anti-allergic action in the air-way and relieve asthma.^{4,5} In the present study, it was found that the whole blood total white cells, EOS and lymphocyte counts increased in the asthma model guinea pigs; and that EOS infiltration significantly decreased in the large dose and middle dose *Wuwei Dihuang* Decoction groups as compared with that of the asthma model group, indicating that *Wuwei Dihuang* Decoction can inhibit inflow of EOS induced by antigens towards the airway.

Researches has demonstrated that IFN- γ is an important cytokine derived by lymphocytes, which are present in many T-cell dependent pulmonary diseases and inhibits the EOS aggregation on the airway mucosa and the production of blood IgE. The results of the present study showed that *Wuwei Dihuang* Decoction can up-regulate serum IFN- γ content of the asthma model guinea pig to the normal level, with a significant difference as compared with the model group. This indicates that *Wuwei Dihuang* Decoction, on the one hand, inhibits the increase of some asthma-inducing inflammatory cytokines, such as interleukine-4 (IL-4), possibly through regulating the Th1/Th2 balance; and on the other hand, it may be related with the inhibitory actions for the EOS aggregation on the airway mucosa and for the production of blood IgE.

LTB4 can stimulate peripheral blood mononuclear leukocytes and T cells to produce IL-1, TNF- α and IL-5,⁶ and it can also increase the IgE syntheses in B cells mediated by the nuclear factor peroxide

proliferative agent-activating receptor- α and IL-4, and up-regulate the expressions of neutrophilic granulocyte CD_{11b}/CD₁₈ desmoprotein and L-selectin.⁷ Therefore, LTB4 can promote syntheses and releases of many kinds of inflammatory cytokines, and the synthesis of LTB4 is regulated by various cytokines, so as to play an important role in asthma cytokines network. In the present experiment, it was found that *Wuwei Dilong* Decoction can decrease the serum LTB4 level in the asthma guinea pigs.

In brief, *Wuwei Dilong* Decoction not only influences the inflammatory cells, such as EOS and lymphocytes, but also participates in the immune regulation to regulate the cytokines, such as LTB4 and IFN- γ . This is possibly one of the mechanisms of *Wuwei Dilong* Decoction for treatment of asthma.

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