NATURAL HERBS TREATMENT ON ALLERGIC BRONCHIAL ASTHMA DISEASE

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ABSTRACT

Objective: The study revealed how Recuperating Lung Decoction (RLD) intervenes in asthma on the basis of successful construction of rabbit asthma model and the effective evaluation guided by Traditional Chinese Medicine (TCM) theory and metabolomics.

Materials and methods: 21 rabbits were randomly divided into three groups: blank control group, model group, treatment group; in 14 of them, the asthma model was established. The rabbits in the treatment group was treated orally with RLD. The rats in the normal and model groups were given 0.9% saline. Metabolites were compared among the groups, which were determined by Gas chromatography-mass spectrometry (GC-MS) methods. Data was analyzed using SAS 8.0.

Results: A total of 7 kinds of metabolites were detected from the Broncho alveolar lavage fluid (BALF) of rabbits, and 4 kinds of metabolites were significant after statistic comparison. The content of some metabolites changed in the model group and treatment group compared with the control group. It could be inferred that the changing was affected by modeling and drug treatment. The increased content of glycine, aspartate and galactose as well as decreased content of myristic acid were closely related to the inflammatory process in bronchial asthma. These 4 metabolites’ levels obviously changed after treatment with Recuperating Lung Decoction.

Conclusion: The study has developed a new Recuperating Lung Decoction intervention model in metabolomic changes of asthma in animal model, and has important theoretical and practical significance with reference to clinical and basic TCM theory.

Key words: Metabolomics, BALF, Asthma, Recuperating Lung Decoction.

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Introduction

Bronchial asthma (asthma) is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role (mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial cells)10. This inflammation makes the body susceptible to various factors that lead to airway hyper-responsiveness and airway narrowing. Improvements in asthma control do not only impact the patients’ daily life but are also associated with a reduced risk of exacerbations and lung function impairment10.

Metabolomic technology, recently developed, is a powerful tool providing standardization for TCM3. The way biological metabolites relate to physiological and pathological changes of the body can be determined4. GC-MS is expected to develop into a powerful tool of metabolomics by means of integral mass spectrometry database, simple operation, and relatively low cost and strong separation analysis capabilities5. As the main method to study the cellular components and soluble components in the alveolar epithelial surface liquid, BALF technology has been applied for a hundred years6.
In this study, we established rabbit model of asthma by injecting and atomizing ovalbumin (OVA). Then we combined the evaluation with metabolic changes in BALF analyzed by GC-MS in order to find possible metabolic markers of asthma.

Materials and methods

**Animal sample and grouping**

21 purebred white sterile New Zealand rabbits, male or female, aged 2 months, weighting approximately 1.5 kg (provided by the Beijing HaidianXingwang experimental animal breeding plant) were randomly divided into three groups. The animals were fed freely.

**Methods of establishing the model with allergic bronchial asthma**

Model group and treatment group rabbits were sensitized by i.p. injection with 10% OVA in 0.9% saline. Blank control group rabbits received equivalent dose of saline only. After 14 days we put rabbits in a closed container which connects with the ultrasonic atomizer of 402A type, exposed model group and treatment group rabbits to an aerosol of 1% OVA in 0.9% saline for 7 days (normal group received saline only).

**Methods of decoction preparation**

All of the herbal pieces of Chinese medicine in RLD were bought from Beijing Tongrentang pieces co., and the extraction of active components was performed by the water-boiling method.

**Bronchoalveolar lavage fluid processing and eosinophil count**

After anesthesia, we fastened the rabbit to a dissecting table, incise the trachea and insert a cannula with another cannula into it, inject 3 ml normal saline and pump back 2–3 times. At last, centrifuge the cell suspension for 0.1ml fluid which was then smeared on the glass slide, let it dry out in the wind for Wright-Giemsa staining about 30 min.

**Metabolomics detection analysis**

To identify the metabolites, we have comprehensively compared the mass spectra of each peak against the National Institute of Standards and Technology (NIST) library and standard library. Partial least squares regression analysis (PLS-DA) was completed with software “MetaboAnalyst 2.0 online”.

(Online Website: http://www.metaboanalyst.ca/ MetaboAnalyst/faces/Home.jsp).

**Statistical methods**

With application of SAS V8 software (SAS Institute Inc.), the metabolite content differences among the three groups were tested with the method of one-way ANOVA analysis, and measurement data was represented by $\overline{x} \pm s$.

**Results**

**Evaluation results of asthma model**

**General states**

Model group: symptoms such as fantod and restlessness, shortness of breath, abdominal muscle spasms, cyanosis of lips and nose, urinary and fecal incontinence and slow movements were observed. In severe cases there was respiratory depression or dysrhythmias, limb collapse and dull reaction. The above mentioned signs and symptoms were not observed in the normal group. HE staining results of rabbit’s lung tissue

Under light microscope, the lung tissue and bronchi of the model group were infiltrated by eosinophils (EOS), and airway ciliated epithelial cell disappeared, with many places fractured and falling off (Figure 1).

Note: (a) and (b) were blank control groups under light microscope (10 times and 20 times). (c) and (d) were model groups under light microscopy (20 times and 40 times).

![Figure 1: HE staining of blank control and model groups.](a) (b) (c) (d)

**Results of BALF eosinophil count**

The EOS counts in model group were higher than that in blank control group, where there was a significant difference between the two groups ($P<0.05$) (See Figure 2).
Note: Each of the groups showed eosinophil content, with expression in the model group being significantly higher than in the blank control group.

**Results of BALF metabolomics**

**Result of PLS-DA score and VIP value**

Score plots results showed that the metabolites in the three samples were clearly separated in the respective quadrants, indicating that there were significant differences in metabolite composition among the groups. This difference was more obvious between the groups put on Recuperating Lung Decoction and the model group. In the loading plot, the more closer the variable to the origin, the less contribution it has; when the variable comes farther, it’s means a greater contribution.

According to the variable importance in the projection (VIP) results, the descending order of the VIP values were glycine, galactose, palmitic acid, ribitol, aspartate and stearic acid. The variables glycine and galactose made most contributions to the classification (See Figure 3).

Note: 0: blank control group; 1: model group; 2: Recuperating Lung Decoction group. (a) overview plot; (b) 2 score plot; (c) loading plot; (d) VIP value of the variables. For PLS-DA cross validation details, R² = 0.79873, Q² = 0.7145, and accuracy = 0.85714. Glycine, for example, was expressed highest in the model group (red box), followed by the blank control group (yellow box), and the Recuperating Lung Decoction group was the lowest (green box).

**Result of Hierarchical Clustering**

Hierarchical clustering is commonly used for unsupervised clustering. Results indicated that the blank control group, model group and Recuperating Lung Decoction group could be easily distinguished (see Figure 4).
Glycine, aspartate, galactose and myristate were different among the three groups, and the P values have been listed in Table 1.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Area (μmol)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>5041.18±2292.02</td>
<td>0.004</td>
</tr>
<tr>
<td>Aspartate</td>
<td>181.11±115.85</td>
<td>0.012</td>
</tr>
<tr>
<td>Ribitol</td>
<td>2123.21±3294.19</td>
<td>0.077</td>
</tr>
<tr>
<td>Galactose</td>
<td>209.52±62.13</td>
<td>0.015</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>2990.29±2837.53</td>
<td>0.442</td>
</tr>
<tr>
<td>Myristate</td>
<td>11093.69±3798.13</td>
<td>0.024</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>9417.88±2630.94</td>
<td>0.172</td>
</tr>
</tbody>
</table>

Table 1: One-way ANOVA results of the peak area.

Discussion

The study in recent years has revealed that glycine can prevent cellular damage due to various toxic substances in the cell, organ and overall level, and also has the effects such as anti-inflammation and immunoregulation. Some study shows that glycine can obviously reduce the coefficient of lung injury, decrease neutrophil aggregation, reduce lipid peroxidation and significantly improve the histological lesions of acute lung injury. The characteristic of glycine has proved that the significant increase of its content in the BALF is related to inflammatory reaction in this experiment. Glycine could inhibit the synthesis of inflammatory cytokines and prevent the secretion of anti-inflammatory cytokines IL-6, TNF-α and factor IL-1 early. On the allergic bronchial asthma rabbit model, the content of glycine increased significantly.

It had many anti-inflammatory effects, such as reducing leukocyte infiltration, inhibiting the synthesis of proinflammatory cytokines and finally blocking the inflammatory process in the end. Glycine is also an important inhibitory neurotransmitter in the central nervous system, and has significant regulating effects on many physiological functions in the human body, especially in genesis and regulation of respiratory rhythm.

Many studies indicated that hypoxia could cause increase in release of excitatory amino acids in the brain, such as aspartic acid. Hypoxia results in a massive release of aspartate, and excessive activation of N-methyl-D-aspartate (NMDA) receptors, which ultimately leads to the neurotoxic effect of the excitatory amino acid.

A study implied that the N-methyl-D-aspartate receptor (NMDAR) was able to decrease hyperoxia-induced lung damage. Researchers used NMDA to perfuse the bronchi and found that it could enhance the resting muscle tone, and contractile effects of acetylcholine and methacholine. NMDAR might have contributed in the experimentally induced airway hyperresponsiveness. Neutrophils are important inflammatory cells, with NMDAR likely affecting inflammation occurrence and degree by mediating neutrophils.

Myristic acid was found to have a significant and positive correlation with serum cholesterol. The study also revealed that tetradecanoic acid could improve the content of the low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). Myristic acid significantly increased in patients with hyperlipidemia, and had a positive correlation with total cholesterol, triglyceride, LDL and apoB levels.

Recently, many studies have shown that there is a correlation between obesity and bronchial asthma, which prompted that overweight or obesity is one of the reasons that caused asthma.

Through orthogonal projection discriminant analysis in BALF of asthmatic mice, Wanxing Eugene Ho found out that energy releasing metabolites decreased and carbohydrates decreased. It was suggested that airway inflammatory diseases consume enormous amount of energy. Significantly, galactose, eosinophil and neutrophil levels had a negative correlation, indicating that these two cells may be associated with reduced galactose levels.
Conclusion

In summary, the expression of glycine and galactose in the model group is closely related to the whole pathologic process of allergic bronchial asthma, especially with respect to anti-inflammatory effects, respiratory inhibition and genetic factors. There is no direct relationship between aspartate and asthma, while over activation of the NMDAR was associated with the occurrence and development of a variety of lung diseases. At the same time, myristic acid possibly perpetuated asthma through its action on blood lipids. Recuperating Lung Decoction played an important role in the repair of metabolic network through these four metabolites.

References


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