Role of histamine in airway remodeling of asthmatic guinea pig

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Abstract: To investigate the role of histamine in airway remodeling, 50 healthy guinea pigs were divided into 5 groups: control group: nebulized inhalation of distilled water for 8 weeks; asthma model group: nebulized inhalation of ovalbumin (OVA) for 8 weeks after sensitization; continued asthma model group: nebulized inhalation of OVA for 14 weeks after sensitization and histamine was added in the last 6 weeks; histamine group: nebulized inhalation of OVA for 14 weeks after sensitization and histamine receptor antagonists were added in the last 6 weeks. For each group, the concentration of histamine, sodium ion (Na+), chlorine ion (Cl−), arterial partial pressure of oxygen (PaO2), arterial partial pressure of carbon dioxide (PaCO2), pH, actual bicarbonate (AB), standard bicarbonate (SB) in serum, and thickness of airway mucosa, base membrane and smooth muscle were measured and compared with each other. The results showed that: (1) the concentration of histamine in serum and the thickness of airway increased, the following order was, the control group, the asthma model group, the continued asthma model group and the histamine group (P<0.01); and the concentration of histamine in serum and the thickness of airway of antagonist group was lower than that of the continued asthma model group (P<0.05, 0.01). (2) PaO2 of the asthma model group was lower than that of the normal control group (P<0.01); PaO2, pH, AB, SB decreased, the following order was, the asthma model group, the continued asthma model group and the histamine group (P<0.01); and PaO2, pH, AB, SB of the antagonist group was higher than that of the continued asthma model group (P<0.01); but for PaCO2, the order was converse (P<0.01); For the concentration of Na+ and Cl− in serum, there was no difference among these groups. It is concluded that: (1) Histamine is one of the mediators in the airway remodeling of asthma. (2) Histamine receptor antagonists may play a role in preventing and treating airway remodeling. (3) There is a negative correlation between the PaO2, pH and the wall thickness of the airway (P<0.01), while a positive correlation between the PaCO2, anion gap (AG) and the wall thickness of the airway (P<0.01).

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Asthma is a chronic inflammatory disease of the airways, characterized by bronchial hyperresponsiveness and obstruction[1,2]. Periodic attacks of asthma may result in smooth muscle hyperplasia, mucus glands hypertrophy, subepithelial fibrosis, angiogenesis and change of extracellular matrix[3]. These pathological changes are called airway remodeling, to which great attention has been paid in recent years for its effects on asthma. Growth factors, cytokines, enzymes and inflammatory mediators all play an important role in airway remodeling[4]. Histamine is one of the earliest found inflammatory mediators. The central role of histamine as a mediator of allergic reaction is unchallenged and is also supported by the efficacy of antihistamines in relieving symptoms of the early-phase allergic response[5]. Marone et al. reported that histamine might contribute to the long-term evolution of lung inflammation and tissue remodeling in allergic diseases in vitro[6]. We speculated that the role of histamine is not limited to the early inflammatory reaction, but also important in the chronic inflammatory response. The present study aimed at the role of histamine in airway remodeling in vivo by detecting the changes of thickness of airway wall and indexes of blood gas and acid-base balance.

1 MATERIALS AND METHODS

1.1 Materials
Ovalbumin (Sigma); Histamine (Shanghai Lizhu Dongfeng); Diphenhydramine injection (Kaifeng Pharmaceutical Company); Ranitidine injection (Hangzhou Minsheng Pharmaceutical Company); Guinea pig (Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology); Model 402 ultrasonic atomizer (Shanghai Siling Medical Equipment Plant); High Performance Liquid Chromatography (HPLC) (Agilent 1100, USA); Image analyzing system (HMIAS-2000); Rapid 248 blood gas analyzer (Abbott Company, USA).

1.2 Division and disposal of guinea pigs
After adaptively fed for one week, 50 healthy male guinea pigs, weighing (250±10) g, were divided into five groups randomly, i.e., A (control group); B (asthma model group); C (continued asthma model group); D (histamine group); E (antagonist group). Each group includes ten guinea pigs. To sensitize, 1 ml 10% ovalbumin (OVA) was injected into the guinea pigs’ abdomen of groups B, C, D and E, while equal volume of distilled water for group A as normal control. Fifteen days later, 1% OVA was nebulized and inhaled by groups B, C, D and E, once for 2~5 min per day; group A was treated in the same way except the OVA was replaced by distilled water.

As a result, guinea pigs in groups B, C, D and E began to cough, sneeze and breathe heavily. On the 70th day, groups A and B were observed and tested in the following indexes. While groups C, D and E were treated with 1% OVA continually. Meanwhile, 0.5 mg/L histamine was nebulized and inhaled by group D, once 4~7 min per day; group E was injected with 5 ml/kg diphenhydramine and 15 mg/kg ranitidine into abdomen twice a day. Group C was injected with 1 ml distilled water into abdomen, twice a day. On the 112th day, the same indexes were observed for guinea pigs in groups C, D and E.

1.3 Observation and test
1.3.1 Observation of the activities and the amount of food eaten by guinea pigs
1.3.2 Testing of the concentration of histamine in serum
On the 70th day, after narcotized by 0.5 ml/kg urethane, blood was taken from carotis communis of guinea pigs in groups A and B by intubation, and the serum concentrations of histamine were tested by HPLC.

1.3.3 Testing of thickness of airway
Specimens from trachea, bronchus, small bronchus (integral mucosa, without cartilage, diameter < 500 μm) were taken respectively, then fixed in formalin, wrapped in paraffin wax, sliced up, and stained with hematoxylin-eosin (HE). The areas and inner girth of mucosa, base membrane and
smooth muscle in trachea, bronchus and small bronchus were tested by image analyzer. Areas-inner girth ratio represented the thickness of each layer[7].

1.3.4 [Na+] and [Cl−] in serum and indexes of blood gas and acid-base

Twenty-four hours after 1% OVA was nebulized and inhaled by guinea pigs for the last time, they were narcotized promptly, intubated through carotic communis and taken blood. PaO2, PaCO2, pH, AB, SB, [Na+] and [Cl−] in serum were tested.

1.3.5 Correlation between thickness of airway and blood gas indexes

Correlation between the thickness of airway in trachea, bronchus, small bronchus, PaO2, PaCO2, pH and anion gap (AG) was investigated.

1.4 Statistical analysis

Data were expressed as mean±SD. t test, analysis of variance, q test and correlation were conducted by using SPSS software.

2 RESULTS

2.1 Ordinary manifestation of guinea pigs

Compared with normal controls, asthmatic guinea pigs moved and ate less, and were easy to breathe heavily and quickly. The severity was group D > group C > group E > group B.

2.2 Serum histamine

The concentrations of histamine in serum were: group A, (0.349 ± 0.071) µg/ml; group B, (0.541 ± 0.028) µg/ml; group C, (0.689 ± 0.049) µg/ml; group D, (0.843 ± 0.056) µg/ml; group E, (0.581 ± 0.051) µg/ml. Comparison of histamine in serum among five groups was shown in Fig. 1.

2.3 Morphological examination on the normal control and the asthma model group

Take bronchiole for example. There were a large number of inflammatory cells in and around bronchiole of the asthma model group and a small part of its mucosa fell off, and the thickness of airway was higher than that in the normal control group. While in the normal control group, only a few inflammatory cells were found in and around bronchiole (Fig. 2, 3).

2.4 Thickness of airway mucosa in each group

Along with increase or decrease of the concentration of histamine in serum, the thickness of airway mucosa acted in the same way (Table 1).
2.5 Thickness of airway base membrane in each group

The changes in the thickness of base membrane were greater in trachea and bronchus than in small bronchus in HE staining. Along with increase or decrease of the concentration of histamine in serum, the thickness of base membrane acted in the same way (Table 2).

2.6 Thickness of airway smooth muscle in each group

Along with increase or decrease of the concentration of histamine in serum, the thickness of smooth muscle acted in the same way (Table 3).

2.7 Changes of $[\text{Na}^+]$, $[\text{Cl}^-]$ in serum and blood gas in each group

Along with increase or decrease of the concentration of histamine in serum, $\text{PaCO}_2$ increased and decreased gradually; but $\text{PaO}_2$ decreased or increased, and the changes of $[\text{Na}^+]$, $[\text{Cl}^-]$ in serum were not obvious (Table 4).

2.8 Changes of acid-base balance in each group

Along with the increase or decrease of the concentration of histamine in serum, $\text{AG}$ increased and decreased gradually; but pH, AB and SB decreased and increased, respectively (Table 5).

2.9 Relationship between thickness of airway and blood gas indexes

There is a negative correlation between the $\text{PaO}_2$, pH and the wall thickness of the airway ($P<0.01$), while a positive correlation between the $\text{PaCO}_2$, AG and the wall thickness of the airway ($P<0.01$) (Table 6).
3 DISCUSSION

The guinea pigs in asthma model group moved and ate less, and were easy to breathe heavily and quickly after moving about. Compared with the normal control group, there were more inflammatory cells, and the thickness of airway mucosa, base membrane and smooth muscle in trachea, bronchus, and small bronchus were all higher. And the longer asthma lasted, the greater the changes. These indicated that asthmatic airway remodeling model had been established successfully.

In vivo, histamine is mainly produced by histidine deacidification, while a little comes from food and bacteria. It is stored predominantly in basophils and hypertrophic cells. Histamine is released upon inflammatory mediators stimulation. As shown in our results, in asthmatic guinea pigs, the concentration of histamine in serum increased noticeably, and the thickness of airway mucosa, base membrane and smooth muscle in trachea, bronchus, small bronchus became thicker evidently, at the same time, PaO₂ became lower, while PaCO₂ and AG became higher, and mixed acid-base imbalance occurred; after inhaled exogenous histamine, the serum histamine became higher, and the airway mentioned above became thicker, the changes of PaO₂, PaCO₂, AG and mixed acid-base imbalance became severer. On the contrary, when histamine H₁ and H₂ receptors were used, the airway mentioned above became thinner, and the changes of PaO₂, PaCO₂, AG and mixed acid-base imbalance became alleviatory. There is a negative correlation between the PaO₂, pH and the wall thickness of the airway, while a positive correlation between the PaCO₂, AG and the wall thickness of the airway. These proved that histamine was one of the mediators in asthmatic airway remodeling. Recently, it has been demonstrated that histamine could stimulate the proliferation of fibroblasts in the cultured oral mucus [9], airway smooth muscle of rabbits in vitro [9] and human articular chondrocytes (HAC) in culture [10]. However, the precise mechanisms of how histamine leads to asthmatic airway remodeling need further investigation. In addition, the changes of blood gas and acid-base balance might have some relationship with hypoventilation and ventilation-perfusion imbalance caused by airway remodeling.

It has been proved that there are three kinds of functionally different histamine receptors in tissues. When histamine acts upon H₁ receptor, inflammation is stimulated; when histamine acts upon H₂ receptor, inflammation is restrained; when histamine acts upon H₃ receptor, produce and release of histamine are restrained. In our study, interdicted the H₁ and H₂ receptors by diphenhydramine and ranitidine, the effect of histamine became weak. As a result, the thickness of airway mucosa, base membrane and smooth muscle in trachea, bronchus, small bronchus became low, decreasing of PaO₂ and increasing of PaCO₂ became alleviatory, and acid-base tended to become balanced. All these indexes showed the improved lung ventilation. Therefore, histamine receptor antagonists might be used as one of the measurements to prevent and treat asthmatic airway remodeling.

It is concluded that histamine is one of the mediators in the airway remodeling of asthma, and histamine receptor antagonists may play a substantial role in preventing and treating airway remodeling. Our research represents an alternative way to explore the mechanisms of asthma, and also provides a base for its prevention and treatment.

REFERENCES

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