

Research Paper

Role of histamine in airway remodeling of asthmatic guinea pig

WANG Xiang-Hong^{1,2}, LIU Sheng-Yuan^{1,*}, CHEN Bao-Sheng³, YU Shang-Bin¹, YE Shi-Qiao¹, CHEN Qi-Ling¹

¹Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China;

²Department of Pathology and Pathophysiology, Medical College; ³Department of Forensic Medicine, Henan University of Science and Technology, Luoyang 471003, China

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; histamine group: nebulized inhalation of OVA for 14 weeks after sensitization and histamine was added in the last 6 weeks; antagonist 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 (PaO_2), arterial partial pressure of carbon dioxide (PaCO_2), 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 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) PaO_2 of the asthma model group was lower than that of the normal control group ($P<0.01$); PaO_2 , 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 PaO_2 , pH, AB, SB of the antagonist group was higher than that of the continued asthma model group ($P<0.01$); but for PaCO_2 , 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 PaO_2 , pH and the wall thickness of the airway ($P<0.01$), while a positive correlation between the PaCO_2 , anion gap (AG) and the wall thickness of the airway ($P<0.01$).

Key words: histamine; asthma; airway remodeling

组胺在哮喘豚鼠气道重塑中的作用

王向红^{1,2}, 刘声远^{1,*}, 陈宝生³, 余上斌¹, 叶仕乔¹, 陈琪玲¹

¹华中科技大学同济医学院病理生理系, 武汉 430030; ²河南科技大学²医学院病理学与病理生理学教研室; ³法医系, 洛阳 471003

摘要: 本研究旨在探讨组胺在哮喘豚鼠气道重塑中的作用。将 50 只健康雄性豚鼠随机分为 5 组。正常对照组: 雾化吸入蒸馏水 8 周; 哮喘模型组: 致敏后雾化吸入卵白蛋白(ovalbumin, OVA) 8 周; 哮喘模型延续组: 致敏后雾化吸入 OVA 14 周; 组胺组: 致敏后雾化吸入 OVA 14 周, 最后 6 周同时加用组胺; 组胺受体拮抗剂组: 致敏后雾化吸入 OVA 14 周, 最后 6 周同时加用组胺受体拮抗剂。检测血清组胺、 Na^+ 、 Cl^- 浓度、动脉血氧分压(PaO_2)、动脉血二氧化碳分压(PaCO_2)、pH、实际碳酸氢盐(actual bicarbonate, AB)、标准碳酸氢盐(standard bicarbonate, SB)以及气道壁粘膜层、基底膜层和平滑肌层厚度。所得结果如下: (1) 哮喘模型组血清组胺浓度、气道壁厚度明显高于正常对照组 ($P<0.01$); 哮喘模型延续组明显高于哮喘模型组($P<0.01$); 组胺组明显高于哮喘模型延续组($P<0.01$); 组胺受体拮抗剂组低于哮喘模型延续组($P<0.05, 0.01$)。 (2) 哮喘模型组 PaO_2 小于正常对照组($P<0.01$); 哮喘模型延续组 PaO_2 、pH、AB、SB 小于哮喘模型组($P<0.01$), 而 PaCO_2 大于哮喘

Received 2005-06-13 Accepted 2005-09-05

*Corresponding author. Tel: +86-27-83693669; Fax: +86-27-83692608; E-mail: Liusy868@sina.com.cn

模型组($P<0.01$)；组胺组 PaO_2 、pH、AB、SB 小于哮喘模型延续组($P<0.01$)，而 PaCO_2 大于哮喘模型延续组($P<0.01$)；组胺受体拮抗剂组 PaO_2 、pH、AB、SB 高于哮喘模型延续组($P<0.01$)，而 PaCO_2 低于哮喘模型延续组($P<0.01$)；血清 Na^+ 、 Cl^- 浓度各组间差异不明显。以上结果提示：(1)组胺在哮喘气道重塑中起介导作用。(2)应用组胺受体拮抗剂对防治哮喘气道重塑有一定作用。(3) PaO_2 、pH 与气管、肺门支气管、肺外周小气道气道壁厚度呈负相关($P<0.01$)，而 PaCO_2 、阴离子间隙(anion gap, AG)值与上述气道壁厚度呈正相关($P<0.01$)。

关键词：组胺；哮喘；气道重塑

中图分类号：Q472; R363

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 $< 500\ \mu\text{m}$) 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. PaO_2 , $PaCO_2$, 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, PaO_2 , $PaCO_2$, pH and anion gap (AG) was investigated.

1.4 Statistical analysis

Data were expressed as mean $\pm$ 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, $(0.349 \pm 0.071) \mu\text{g/ml}$; group B, $(0.541 \pm 0.028) \mu\text{g/ml}$; group C, $(0.689 \pm 0.049) \mu\text{g/ml}$; group D, $(0.843 \pm 0.056) \mu\text{g/ml}$; group E, $(0.581 \pm 0.051) \mu\text{g/ml}$. Comparison of histamine in serum among five groups was shown in Fig. 1.

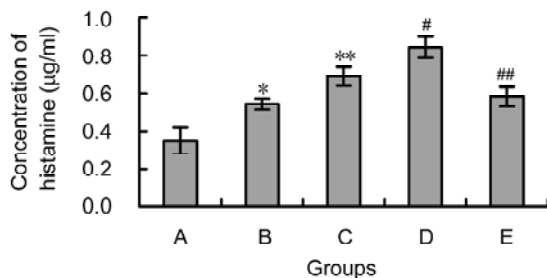


Fig. 1. Comparison of histamine in serum among each group ($\mu\text{g/ml}$, mean $\pm$ SD, $n=9$). A, control group; B, asthma model group; C, continued asthma model group; D, histamine group; E, histamine receptor antagonist group. * $P<0.01$ vs A, ** $P<0.01$ vs B, # $P<0.01$ vs C, ## $P<0.05$ vs C.

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).

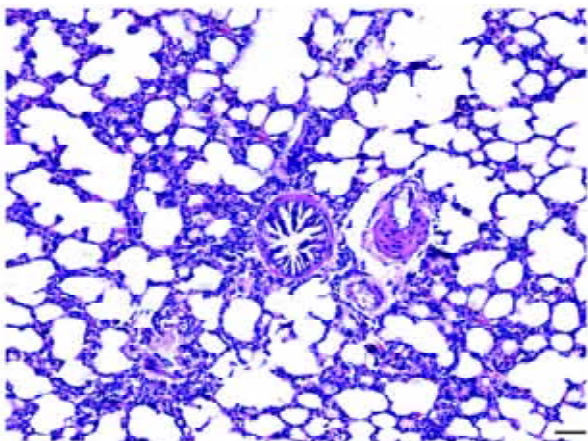


Fig. 2. Bronchiole of control group (HE stain. Scale bar, 50 μm). There were a few inflammatory cells in and around bronchiole wall. Proliferation of smooth muscle was not found.

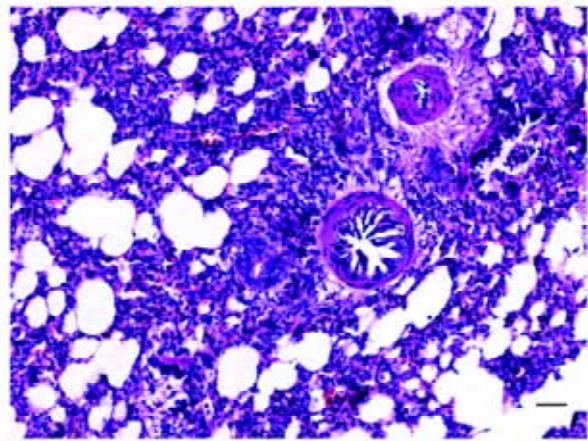


Fig. 3. Bronchiole of asthma model group (HE stain. Scale bar, 50 μm). There were plenty of inflammatory cells in and around bronchiole wall. Significant proliferation of smooth muscle was found.

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).

Table 1. Comparison of thickness of airway mucosa in guinea pigs

Groups	Trachea (μm)	Bronchus (μm)	Small bronchus (μm)
A	22.90 $\pm$ 3.42	23.01 $\pm$ 2.35	7.48 $\pm$ 1.95
B	39.76 $\pm$ 3.98*	39.06 $\pm$ 2.23*	11.79 $\pm$ 1.87*
C	54.02 $\pm$ 3.84**	52.13 $\pm$ 2.98**	14.88 $\pm$ 1.35**
D	61.02 $\pm$ 2.91 [#]	62.78 $\pm$ 3.02 [#]	17.62 $\pm$ 1.23 [#]
E	46.50 $\pm$ 2.83 ^{##}	45.31 $\pm$ 2.29 ^{##}	12.01 $\pm$ 0.80 ^{##}

mean $\pm$ SD, $n=9$. A, control group; B, asthma model group; C, continued asthma model group; D, histamine group; E, histamine receptor antagonist group. * $P<0.01$ vs A, ** $P<0.01$ vs B, [#] $P<0.01$ vs C, ^{##} $P<0.01$ vs C.

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).

Table 2. Comparison of thickness of base membrane in guinea pigs

Groups	Trachea (μm)	Bronchus (μm)
A	6.54 $\pm$ 1.93	7.09 $\pm$ 1.34
B	10.86 $\pm$ 1.07*	11.50 $\pm$ 1.49*
C	15.13 $\pm$ 2.12**	16.42 $\pm$ 1.64**
D	18.89 $\pm$ 1.91 [#]	19.85 $\pm$ 1.73 [#]
E	12.41 $\pm$ 1.26 ^{##}	13.55 $\pm$ 1.83 ^{##}

mean $\pm$ SD, $n=9$. A, control group; B, asthma model group; C, continued asthma model group; D, histamine group; E, histamine receptor antagonist group. * $P<0.01$ vs A, ** $P<0.01$ vs B, [#] $P<0.01$ vs C, ^{##} $P<0.01$ vs C.

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, PaCO_2 increased and decreased gradually; but 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, AG increased and decreased

Table 3. Comparison of thickness of smooth muscle in guinea pigs

Groups	Trachea (μm)	Bronchus (μm)	Small bronchus (μm)
A	20.96 $\pm$ 6.54	25.55 $\pm$ 7.96	21.76 $\pm$ 4.51
B	34.90 $\pm$ 4.75*	37.75 $\pm$ 4.01*	40.95 $\pm$ 6.22*
C	43.07 $\pm$ 4.18**	45.24 $\pm$ 4.31**	55.07 $\pm$ 5.21**
D	48.49 $\pm$ 3.79 [#]	49.95 $\pm$ 2.91 [#]	62.11 $\pm$ 5.29 [#]
E	38.56 $\pm$ 3.19 ^{##}	40.85 $\pm$ 3.07 ^{##}	49.56 $\pm$ 3.27 ^{##}

mean $\pm$ SD, $n=9$. A, control group; B, asthma model group; C, continued asthma model group; D, histamine group; E, histamine receptor antagonist group. * $P<0.01$ vs A, ** $P<0.01$ vs B, [#] $P<0.01$ vs C, ^{##} $P<0.01$ vs C.

Table 4. Changes of $[\text{Na}^+]$, $[\text{Cl}^-]$, blood gas in guinea pigs

Groups	$[\text{Na}^+]$ (mmol/L)	$[\text{Cl}^-]$ (mmol/L)	PaO_2 (mmHg)	PaCO_2 (mmHg)
A	129.90 $\pm$ 1.45	100.17 $\pm$ 1.36	94.80 $\pm$ 3.302	40.61 $\pm$ 1.01
B	130.46 $\pm$ 2.12	101.04 $\pm$ 1.47	79.50 $\pm$ 3.54*	41.21 $\pm$ 1.04
C	129.53 $\pm$ 2.43	99.57 $\pm$ 1.71	61.54 $\pm$ 1.72**	54.40 $\pm$ 2.89**
D	130.23 $\pm$ 2.35	101.22 $\pm$ 1.54	45.55 $\pm$ 6.83 [#]	68.32 $\pm$ 1.95 [#]
E	130.01 $\pm$ 2.07	101.11 $\pm$ 1.41	70.93 $\pm$ 1.93 ^{##}	46.69 $\pm$ 1.98 ^{##}

mean $\pm$ SD, $n=9$. A, control group; B, asthma model group; C, continued asthma model group; D, histamine group; E, histamine receptor antagonist group. * $P<0.01$ vs A, ** $P<0.01$ vs B, [#] $P<0.01$ vs C, ^{##} $P<0.01$ vs C.

Table 5. Changes of acid-base balance in guinea pigs

Groups	pH	AB (mmol/L)	SB (mmol/L)	AG (mmol/L)
A	7.34 $\pm$ 0.01	21.73 $\pm$ 1.36	21.53 $\pm$ 0.65	8.00 $\pm$ 0.25
B	7.30 $\pm$ 0.02	20.22 $\pm$ 0.57	19.71 $\pm$ 0.65	9.20 $\pm$ 0.36
C	7.22 $\pm$ 0.03**	16.76 $\pm$ 2.00**	12.25 $\pm$ 1.67**	13.20 $\pm$ 0.76**
D	7.15 $\pm$ 0.02 [#]	12.91 $\pm$ 1.29 [#]	7.62 $\pm$ 1.53 [#]	16.10 $\pm$ 0.47 [#]
E	7.27 $\pm$ 0.01 ^{##}	18.24 $\pm$ 1.36 ^{##}	17.15 $\pm$ 1.28 ^{##}	10.40 $\pm$ 0.73 ^{##}

mean $\pm$ SD, $n=9$. A, control group; B, asthma model group; C, continued asthma model group; D, histamine group; E, histamine receptor antagonist group. ** $P<0.01$ vs B, [#] $P<0.01$ vs C, ^{##} $P<0.01$ vs C.

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 PaO_2 , pH and the wall thickness of the airway ($P<0.01$), while a positive correlation between the PaCO_2 , AG and the wall thickness of the airway ($P<0.01$) (Table 6).

Table 6. Relationship between thickness of airway, arterial blood gas and acid-base balance (n=45)

	Trachea		Bronchus		Small bronchus	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
PaO ₂	-0.6743	<0.01	-0.6931	<0.01	-0.7214	<0.01
PaCO ₂	0.5709	<0.01	0.5842	<0.01	0.6397	<0.01
pH	-0.5814	<0.01	-0.6043	<0.01	-0.6157	<0.01
AG	0.6078	<0.01	0.6347	<0.01	0.6978	<0.01

PaO₂, arterial partial pressure of oxygen; PaCO₂, arterial partial pressure of carbon dioxide; AG, anion gap.

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 decarboxylation, 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 antagonists 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^[8], 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

- 1 Fahy JV, Corry DB, Boushey HA. Airway inflammation and remodeling in asthma. *Curr Opin Pulm Med* 2000; 6(1): 15-20.
- 2 Ren YH, Qin XQ, Guan CX, Luo ZQ, Zhang CQ, Sun XH. Temporal and spatial distribution of VIP, CGRP and their receptors in the development of airway hyperresponsiveness in the lungs. *Acta Physiol Sin (生理学报)* 2004; 56(2): 137-146.
- 3 Beasley R, Page C, Lichtenstein L. Airways remodeling in asthma. *Clin Exp All Rev* 2002; 2 (4): 109-116.
- 4 Tschumperlin DJ, Drazen JM. Mechanical stimuli to airway

- remodeling. *Am J Respir Crit Care Med* 2001; 164(10 Pt 2): S90-S94.
- 5 Marone G. Milestones in the biology and pharmacology of H₁-receptor antagonists. *Allergy* 1997; 52 (34 Suppl.): 7-13.
 - 6 Marone G, Gentile M, Petraroli A, De Rosa N, Triggiani M. Histamine-induced activation of human lung macrophages. *Int Arch Allergy Immunol* 2001; 124(1-3): 249-252.
 - 7 Opazo Saez A, Du T, Wang NS, Martin JG. Methacholine-induced bronchoconstriction and airway smooth muscle cells in the guinea pig. *J Appl Physiol* 1996; 80(2): 437-444.
 - 8 Su K (苏葵), Liu SF, Shen ZH, Peng LX, Guo JX. Morphologic study of histamine promoting fibroblast cells. *J Clin Stomatol (临床口腔医学杂志)* 1997; 13(3): 132-133 (Chinese, English abstract).
 - 9 Qi HW (戚好文), Liu ZQ, Li HZ, Liao JJ, Liu YG. Effects of histamine on the proliferation of ASMA in rabbits. *Chin J Pathol (中国病理生理学杂志)* 1999; 15(3): 236-239 (Chinese, English abstract).
 - 10 Tetlow LC, Woolley DE. Histamine stimulates the proliferation of human articular chondrocytes *in vitro* and is expressed by chondrocytes in osteoarthritic cartilage. *Ann Rheum Dis* 2003; 62(10): 991-994.