

# Original article

## Inflammatory airway features and hypothalamic-pituitary-adrenal axis function in asthmatic rats combined with chronic obstructive pulmonary disease

CAI Cui, ZHANG Hong-ying, LE Jing-jing, DONG Jing-cheng, CUI Yan, XU Chang-qing, LIU Bao-jun, WU Jin-feng, DUAN Xiao-hong and CAO Yu-xue

**Keywords:** *chronic obstructive pulmonary disease; bronchial asthma; inflammatory airway disease; hypothalamic-pituitary-adrenal axis*

**Background** Bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD) are both inflammatory airway diseases with different characteristics. However, there are many patients who suffer from both BA and COPD. This study was to evaluate changes of inflammatory airway features and hypothalamic-pituitary-adrenal (HPA) axis function in asthmatic rats combined with COPD.

**Methods** Brown Norway (BN) rats were used to model the inflammatory airway diseases of BA, COPD and COPD+BA. These three models were compared and evaluated with respect to clinical symptoms, pulmonary histopathology, airway hyperresponsiveness (AHR), inflammatory cytokines and HPA axis function.

**Results** The inflammatory airway features and HPA axis function in rats in the COPD+BA model group were greatly influenced. Rats in this model group showed features of the inflammatory diseases BA and COPD. The expression of inflammatory cytokines in this model group might be up or downregulated when both disease processes are present. The levels of corticotrophin releasing hormone mRNA and corticosterone in this model group were both significantly decreased than those in the control group ( $P < 0.05$ ).

**Conclusions** BN rat can be used as an animal model of COPD+BA. By evaluating this animal model we found that the features of inflammation in rats in this model group seem to be exaggerated. The HPA axis functions in rats in this model group have been disturbed or impaired, which is prominent at the hypothalamic level.

*Chin Med J 2010;123(13):1720-1726*

Bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD) are the most common inflammatory airway diseases. However, the distinction between them can be blurred, especially in asthmatic patients who smoke or in acute exacerbations of patients of COPD. We have temporarily named this condition COPD+BA. Unfortunately, there is few animal models to study COPD+BA and published studies of COPD+BA were not found. New research has shown that the manifestations of COPD include a number of endocrine disorders, affecting the pituitary, gonads and adrenal glands.<sup>1</sup> Prior studies have focused on the function of the hypothalamic-pituitary-adrenal (HPA) axis, which is the major component of the stress system in patients with BA or COPD. However, the conclusions often vary widely.<sup>2-4</sup> In this study, we attempted to use Brown Norway (BN) rats to establish BA, COPD and COPD+BA animal models and to evaluate these animal models with respect to clinical symptoms, pulmonary histopathology, airway hyperresponsiveness (AHR), inflammatory cytokines and the HPA axis function. The purpose of our work was to provide further insights into the features of these inflammatory airway diseases, especially COPD+BA.

### METHODS

#### Animal

Totally 48 Specific pathogen-free male BN rats (180

g–220 g) were obtained from the Experimental Animal Tech Co. of Weitonglihua (Beijing, China). The animals were housed in the experimental animal center of Fudan University in a 12 hour light-dark cycle, where food and water were continuously available. All procedures and experiments were carried out in accordance with the internationally accepted guidelines for the care and use of laboratory animals and were approved by the Local Institutional Animal Care and Use Committee.

#### Agents and equipment

Ovalbumin (OVA) was from Lizhudongfeng Biological Technology Co. Ltd, Shanghai, China. Aluminum

DOI: 10.3760/cma.j.issn.0366-6999.2010.13.020

Lab of Integrative Medicine for Lung, Inflammation and Cancers, Huashan Hospital, Fudan University, Shanghai 200040, China (Cai C, Zhang HY, Le JJ, Dong JC, Cui Y, Xu CQ, Liu BJ, Wu JF, Duan XH and Cao YX)

Correspondence to: Prof. DONG Jing-cheng, Lab of Integrative Medicine for Lung, Inflammation and Cancers, Huashan Hospital, Fudan University, Shanghai 200040, China (Tel: 86-21-52888301. Fax: 86-21-52888265. Email: jcdong2004@126.com)

Dr. CAI Cui and Dr. ZHANG Hong-ying contributed equally to the present study.

This research was supported by grants from National Basic Science Program of China (No. 2009CB523000); Shanghai Science and Technology Committee (No. 09XD1400700) and China Ministry of Education (No. 200802461053).

hydroxide gel was from Sigma, USA. Sodium pentobarbitone was purchased from the Reagent Company, Shanghai (imported by respective package, USA). The kits for IL-4, IL-5, IL-6, IL-8, TNF- $\alpha$  and IFN- $\gamma$  were from Biosource, USA. The kit for corticosterone (CORT) detection was from R&D company, USA. The radioimmunoassay (RIA) kit for adrenocorticotrophic hormone (ACTH) detection was purchased from the Institute of Neurobiology of Second Military Medical University, China. The SN-695B radioimmunoassay gamma counter was purchased from the Langrihuan Instrument Factory of Nucleus Research (Shanghai, China). The DL-8R refrigerated centrifuge was purchased from the Institute of Centrifuge Mechanism (Shanghai, China). The Gene Amp PCR System of Ferotec Gradien was purchased from Dahe Thermal and Magnetic Electron CO. Ltd, Hangzhou, China. The DYY-8 electrophoresis apparatus was from the Qite Analytical Apparatus Co. Ltd, Shanghai, China. The H6-1 microelectrophoresis bath was from Jingyi Plexiglass Ware Instrument Factory (Shanghai, China). The inverted microscope was from Leica Germany. The Y S9801 medical ultrasonic atomizer was from the Yisheng Technology Co. Ltd, Shanghai, China. The respirator for small animals was from Aoerkete Biological Technology Co. Ltd, Shanghai, China. The Rotor-Gene 3000 real-time PCR was purchased from Corbett Research, Australia. Software for primer design was from Primer Premier 5.0. The device for inducing asthma was a self-made plexiglass case with a volume of 28 cm  $\times$  15 cm  $\times$  13 cm. The smoking apparatus was a self-made fully blocked wooden case with a volume of 60 cm  $\times$  80 cm  $\times$  100 cm.

## Models

### *BA model group*

Twelve BN rats were sensitized on day one with an intraperitoneal injection of 100 mg OVA and 1 ml aluminum hydroxide gel. The rats were challenged once daily for 20 minutes from day 15 to day 21 and again from day 29 to day 35 with 5% OVA saline (5 L/min). The saline-treated control group underwent an identical schedule for sham sensitization and challenge, but received only saline.

### *COPD model group*

Twelve on days one and fourteen, rats were placed supinely and received 0.2 ml of 1  $\mu$ g/ml lipopolysaccharide (LPS) by endotracheal instillation. From day 2 to day 13 and again from day 29 to day 35, rats were treated with cigarette smoke for 40 minutes twice a day.

### *COPD+BA model group*

Twelve BN rats were sensitized on day one with an intraperitoneal injection of 100 mg OVA and 1 ml aluminum hydroxide gel. Then rats underwent endotracheal instillation on day 7 and day 21 with 0.2 ml of 1  $\mu$ g/ml LPS. From day 8 to day 34, the rats were treated with cigarette smoke for 40 minutes twice a day. The rats were challenged with 5% OVA saline (5 L/min)

for 20 minutes once daily before smoking from day 14 to day 20 and again from day 28 to day 34. The entire procedure was completed in five weeks.

### *Endotracheal instillation of LPS*

Twelve BN rats were anesthetized with 2% sodium pentobarbitone (0.3 ml–0.4 ml). An intubated needle was inserted into the trachea and 0.2 ml LPS (1  $\mu$ g/ml) was instilled into the trachea. Successful instillation was indicated by symptoms of tachypnea and tachycardia. Animals were woken within one hour of instillation.

### **Pulmonary histopathology**

The lungs were excised, fixed in 10% formalin and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin (HE). Slides were observed on an inverted microscope.

### **Measurement of AHR**

Methacholine (Mch) at different dosages (0.125 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg) was injected into the jugular vein of the rats via an intubated needle. Airway resistance and tidal volume were observed 4 minutes after Mch challenge.

### **Blood collection**

BN rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbitone (60 mg/kg). Blood was collected using immediate cardiac puncture into heparin anticoagulant tubes and serum tubes for laboratory analysis. The anticoagulant tubes were centrifuged at 3500 r/min for 20 minutes at 4°C. The serum tubes were centrifuged at 1500 r/min for 15 minutes within 2 hours of collection. The plasma and serum were placed in centrifuge tubes and stored in a freezer at –80°C.

### **Measurement of inflammatory cytokines and CORT**

Enzyme Linked Immunosorbent Assay (ELISA) was used to determine the serum concentration of cytokines and CORT according to the instructions. Using CORT as an example: 100  $\mu$ l of serum samples and 50  $\mu$ l of corticosterone antibodies were added to 96-well plates and incubated at 37°C for 2 hours. The plates were washed 3 times and 50  $\mu$ l of substrate was added. The reaction was arrested with a stop solution and absorbance measured at 450 nm.

### **Measurement of ACTH**

RIA was used to test the concentration of ACTH according to the instructions.

### **Measurement of corticotropin releasing hormone (CRH) mRNA**

The CRH mRNA levels were determined by real-time PCR. Hypothalamus (50 mg–100 mg) from BN rats were used for RNA isolation. First, 1 ml of Trizol was added to the plate to lyse the tissue. Then chloroform was added and RNA collected in the aqueous phase after centrifugation. Finally, RNA was precipitated by isopropyl-alcohol, then washed and dissolved in DEPC-treated water. The

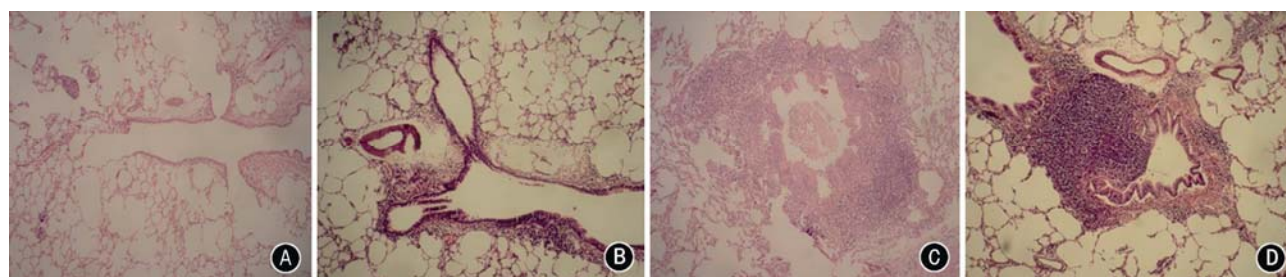


**Figure 1.** Hematoxylin and eosin staining of lung tissues (Original magnification  $\times 200$ ). **A:** The control group. **B:** BA model group. Eosinophils were markedly increased in the bronchial cavity. **C:** COPD model group. Neutrophils and lymphocytes were increased in the bronchial cavity. **D:** COPD+BA model group. The histopathology of this group was similar to that of the COPD model group. However, eosinophils were increased in the bronchial cavity.

**Table 1.** Comparison of resistance among four groups

| Groups  | Methacholine (mg/kg)         |                                 |                                |                                 |                                |
|---------|------------------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------------|
|         | 0.125                        | 0.25                            | 0.5                            | 1                               | 2                              |
| COPD+BA | 1.16 $\pm$ 3.90**            | 2.35 $\pm$ 0.43 <sup>†‡</sup>   | 3.01 $\pm$ 0.02 <sup>†‡</sup>  | 3.52 $\pm$ 0.03 <sup>†§</sup>   | 4.07 $\pm$ 0.38 <sup>†‡</sup>  |
| COPD    | 1.23 $\pm$ 0.62**            | 1.47 $\pm$ 0.16***              | 2.01 $\pm$ 0.28 <sup>†§§</sup> | 1.99 $\pm$ 0.4 <sup>†§††</sup>  | 2.07 $\pm$ 0.43 <sup>†§§</sup> |
| BA      | 0.51 $\pm$ 0.12 <sup>‡</sup> | 3.00 $\pm$ 0.43 <sup>†‡</sup>   | 3.51 $\pm$ 0.17 <sup>†‡</sup>  | 4.01 $\pm$ 0.13 <sup>†§</sup>   | 4.27 $\pm$ 0.28 <sup>†‡</sup>  |
| Control | 0.31 $\pm$ 0.12 <sup>‡</sup> | 0.25 $\pm$ 0.38 <sup>§†††</sup> | 0.3 $\pm$ 0.13 <sup>§†††</sup> | 0.26 $\pm$ 0.38 <sup>§†††</sup> | 0.26 $\pm$ 0.5 <sup>§†††</sup> |

\* $P < 0.05$ , <sup>†</sup> $P < 0.01$  compared with the control group. <sup>‡</sup> $P < 0.05$ , <sup>§</sup> $P < 0.01$  compared with the BA group. <sup>‡</sup> $P < 0.05$ , <sup>†</sup> $P < 0.01$  compared with the COPD group. \*\* $P < 0.05$ , <sup>††</sup> $P < 0.01$  compared with the COPD+BA group.

concentrations of RNA samples were measured by a spectrophotometer to determine the OD260 and OD260/280 values. cDNA was synthesized from 3  $\mu$ g of isolated RNA in RT buffer containing 2.5 mmol/L dNTPs, 0.5  $\mu$ g anchored oligo-dT, 1  $\mu$ l RNasin, and 1  $\mu$ l M-MML reverse transcriptase. The resulting cDNA pool was used for real-time PCR. Primers (designed by Primer 5.0) for CRH were 5'-GTGGCTCTGTCGCCCTGTCT-3'/5'-AGCAGCGGGACTTCTGTTGA-3' and primers for  $\beta$ -actin were 5'-CCTGTACGCCAACACAGTGC-3'/5'-ATACCCTGCTTGCTGCTGATCC-3'. Real-time PCR was done with 40 cycles. The conditions of cycling parameters were 5 minutes at 95°C, 15 seconds at 95°C, 15 seconds at 60°C, 20 seconds at 72°C, 15 seconds at 85.5°C, and finally a melting curve of 2 minutes at 95°C, 20 seconds at 60°C, and 20 seconds at 95°C.

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using the SPSS software and analysis of variance (ANOVA). Differences were considered statistically significant if  $P < 0.05$ .

## RESULTS

### Clinical symptoms

#### BA model group

Sneeze, cough, tachypnea, mouth breathing, opisthotonos, withered hair, and emaciation were seen.

#### COPD model group

Cough, tachypnea, opisthotonos, slackened skin, anorexia, depression, sluggishness, withered hair and a thin and small body were seen.

#### COPD+BA model group

Cough, tachypnea, wheezing, rales, increase of secretions,

anorexia, withered hair and a thin and small body were seen.

### Pulmonary histopathology

#### Observations

The counts of lymphocytes and eosinophils, infiltration of inflammatory cells in the bronchus and alveolar space, bronchial submucosal edema and hyperplasia of smooth muscle were determined.

#### BA model group

Compared to the control group (Figure 1A), the main airway of the BA model group showed inflammatory changes. Infiltrating inflammatory cells, especially eosinophils, were markedly increased in the bronchial cavity, with concurrent bronchial submucosal edema and hyperplasia of smooth muscle (Figure 1B).

#### COPD model group

Compared to the control group, the pulmonary parenchyma of the COPD model group showed inflammatory changes. Infiltrating inflammatory cells, especially neutrophils and lymphocytes, were increased in the bronchial cavity, with concurrent bronchial hyperplasia of smooth muscle (Figure 1C).

#### COPD+BA model group

The histopathology of the COPD+BA model group was similar to that of the COPD model group. However, the infiltration was comprised of eosinophils and made up diffuse lesions (Figure 1D).

### AHR results

Expiratory resistance (Re) was the observational index. Compared with the control group, the Re of all three model groups were significantly increased (Table 1). The Re of the control group showed no significant change

with an increase of Mch concentration, while Re of the COPD model group was only increased in early stimulation. The Res of the other two model groups, especially of the BA model group, were increased with increasing Mch concentrations (Figure 2).

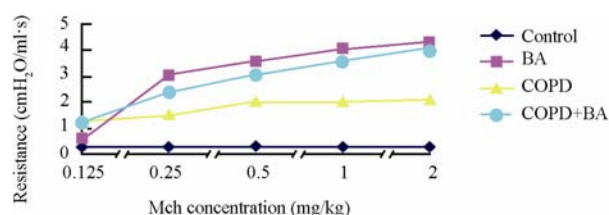


Figure 2. The changes of expiratory resistance of BN rats.

### Changes of inflammatory cytokines

Compared to the control group, the IL-4 and IL-6 serum concentrations in the COPD model group were both significantly decreased ( $P < 0.05$ ,  $P < 0.01$ ), while the serum IL-5 concentration in the same group was significantly increased ( $P < 0.05$ ). The serum IFN- $\gamma$  concentration in the COPD+BA model group was significantly decreased ( $P < 0.01$ ), while the serum TNF- $\alpha$  concentration in the BA model group was significantly increased ( $P < 0.05$ ). The serum IL-8 concentrations in the BA and COPD+BA model groups were both significantly increased ( $P < 0.01$ ) while that in the COPD model group was significantly decreased ( $P < 0.01$ ).

Compared with the BA model group, the IL-4 and IL-6 serum concentrations in the COPD model group were significantly decreased ( $P < 0.05$ ,  $P < 0.01$ ), while the serum IL-5 concentration of the same group was significantly increased ( $P < 0.05$ ). The serum IFN- $\gamma$  concentration in the COPD+BA model group was significantly decreased ( $P < 0.01$ ). The serum TNF- $\alpha$  concentrations in the control group and the COPD+BA model group were both significantly decreased ( $P < 0.05$ ). The serum IL-8 concentrations in the control group and the COPD model group were both significantly decreased ( $P < 0.01$ ), while that in the COPD+BA model group was significantly increased ( $P < 0.01$ ).

Compared with the COPD model group, the serum IL-4 concentrations of the other three groups were all significantly increased ( $P < 0.01$ ), and that in the BA model group was the highest in four groups. The serum IL-5 concentrations of the other three groups were all significantly decreased ( $P < 0.05$ ,  $P < 0.01$ ). The serum IL-6 concentrations in the control group and the BA model group were both significantly increased ( $P < 0.05$ ).

The serum TNF- $\alpha$  concentration in the COPD+BA model group was significantly decreased ( $P < 0.05$ ), and the serum IL-8 concentrations of the other three groups were all significantly increased ( $P < 0.01$ ).

Compared with the COPD+BA model group, the serum IL-4 concentration in the COPD model group was significantly decreased ( $P < 0.01$ ), while the serum IL-5 concentration in this model group was significantly increased ( $P < 0.01$ ). The serum IFN- $\gamma$  concentrations of the other three groups were all significantly increased ( $P < 0.01$ ). The serum TNF- $\alpha$  concentrations in the BA and COPD model groups were both significantly increased ( $P < 0.05$ ), and the serum IL-8 concentrations of the other three groups were all significantly decreased ( $P < 0.01$ ) (Table 2).

### Changes of HPA axis function

Compared with the control group, the CRH mRNA and serum CORT concentrations of the COPD and COPD+BA model groups were all significantly decreased ( $P < 0.05$ ,  $P < 0.01$ ), while those of the BA model group were higher.

Compared with the BA model group, the CRH mRNA and serum CORT concentrations of the COPD and COPD+BA model groups were all significantly decreased ( $P < 0.05$ ,  $P < 0.01$ ).

Compared with the COPD model group, the CRH mRNA concentrations of the control group and the BA model group were both significantly increased ( $P < 0.05$ ). The serum CORT concentrations of the other three groups were also significantly increased ( $P < 0.05$ ,  $P < 0.01$ ).

Compared with the COPD+BA model group, the CRH mRNA concentrations of the control group and the BA model group were both significantly increased ( $P < 0.05$ ). The serum CORT concentrations of the control group and the BA model group were also significantly increased ( $P < 0.05$ ), while that of the COPD model group was significantly decreased ( $P < 0.05$ ). There was no significant difference of the plasma ACTH concentrations among these four groups (Table 3).

## DISCUSSION

BA and COPD are the most common chronic inflammatory airway diseases. Epidemiological data has suggested that the incidences of both diseases are increasing throughout the world. The prevalence of BA in

Table 2. Comparison of inflammatory cytokines among four groups

| Groups  | n  | IL-4 (pg/ml)                    | IL-5 (pg/ml)                    | IL-6 (pg/ml)                  | IFN- $\gamma$ (pg/ml)          | TNF- $\alpha$ (pg/ml)           | IL-8 (pg/ml)                       |
|---------|----|---------------------------------|---------------------------------|-------------------------------|--------------------------------|---------------------------------|------------------------------------|
| COPD+BA | 11 | 39.33 $\pm$ 7.84 <sup>†</sup>   | 18.03 $\pm$ 4.67 <sup>†</sup>   | 1.38 $\pm$ 0.04               | 16.14 $\pm$ 2.09 <sup>†§</sup> | 28.71 $\pm$ 5.21 <sup>‡</sup>   | 107.71 $\pm$ 110.35 <sup>†§¶</sup> |
| COPD    | 12 | 25.46 $\pm$ 6.11 <sup>†§†</sup> | 36.11 $\pm$ 3.36 <sup>†††</sup> | 1.13 $\pm$ 0.63 <sup>**</sup> | 27.39 $\pm$ 9.34 <sup>††</sup> | 35.20 $\pm$ 10.79 <sup>**</sup> | 42.86 $\pm$ 9.17 <sup>†§††</sup>   |
| BA      | 12 | 45.71 $\pm$ 14.22 <sup>†</sup>  | 20.17 $\pm$ 5.74 <sup>‡</sup>   | 1.57 $\pm$ 0.09 <sup>‡</sup>  | 28.49 $\pm$ 6.41 <sup>††</sup> | 37.05 $\pm$ 6.50 <sup>***</sup> | 76.00 $\pm$ 20.70 <sup>†††</sup>   |
| Control | 12 | 40.66 $\pm$ 22.52 <sup>†</sup>  | 21.02 $\pm$ 8.98 <sup>‡</sup>   | 1.61 $\pm$ 0.05 <sup>‡</sup>  | 30.98 $\pm$ 4.06 <sup>††</sup> | 30.00 $\pm$ 12.19 <sup>‡</sup>  | 58.32 $\pm$ 11.46 <sup>§†††</sup>  |

<sup>†</sup> $P < 0.05$ , <sup>‡</sup> $P < 0.01$  compared with the control group. <sup>‡</sup> $P < 0.05$ , <sup>§</sup> $P < 0.01$  compared with the BA group. <sup>‡</sup> $P < 0.05$ , <sup>¶</sup> $P < 0.01$  compared with the COPD group. <sup>\*\*</sup> $P < 0.05$ , <sup>††</sup> $P < 0.01$  compared with the COPD+BA group.

**Table 3.** Comparison of HPA axis function among four groups

| Groups  | n  | CRH mRNA                  | n  | ACTH (pg/ul)            | n  | CORT (ng/ml)                   |
|---------|----|---------------------------|----|-------------------------|----|--------------------------------|
| COPD+BA | 9  | 0.81±0.12 <sup>**</sup>   | 11 | 2.01±0.17 <sup>NS</sup> | 9  | 1540±186.91 <sup>**  </sup>    |
| COPD    | 7  | 1.99±1.11 <sup>**</sup>   | 7  | 2.07±0.07 <sup>NS</sup> | 7  | 1155.73±332.60 <sup>†§**</sup> |
| BA      | 12 | 5.02±1.51 <sup>†**</sup>  | 8  | 2.19±0.25 <sup>NS</sup> | 12 | 1777.84±263.09 <sup>**</sup>   |
| Control | 8  | 4.46±1.33 <sup>  **</sup> | 8  | 2.19±0.20 <sup>NS</sup> | 8  | 1720.33±378.04 <sup>†**</sup>  |

<sup>\*</sup>P <0.05, <sup>†</sup>P <0.01 compared with the control group. <sup>‡</sup>P <0.05, <sup>§</sup>P <0.01 compared with the BA group. <sup>||</sup>P <0.05, <sup>†</sup>P <0.01 compared with the COPD group. <sup>\*\*</sup>P <0.05 compared with the COPD+BA group. NS: not significant.

developed countries is approximately 10% in adults and even higher in children, while the prevalence in developing countries is lower but increasing rapidly. The global prevalence of COPD is approximately 10% among individuals over the age of 40.<sup>5</sup>

BA and COPD have some similarities, such as airflow obstruction, pulmonary inflammation and AHR.<sup>6</sup> Sometimes the distinction between BA and COPD becomes blurred, especially in asthmatic subjects who smoke or in acute exacerbations of patients with COPD. For this reason, we have temporarily named this condition COPD+BA. However, there is little research concerning COPD+BA reported in either clinical or in basic medicine. We had already considered using a cigarette smoke-induced method to establish a COPD animal model.<sup>7</sup> The rats would then be challenged and divided into two groups according to their AHR. The rats had the features of AHR and it seemed to be a successful animal model of COPD+BA. This procedure appeared to be the closest to COPD+BA, but it required months of cigarette smoke exposure. Therefore, our study used BN rats as experimental animals, sensitized and challenged with OVA, in addition to LPS instilled and fumed to establish the COPD+BA animal model. The process included the simultaneous induction of BA and COPD, and was the first step for further study of COPD+BA.

From our study, we found that the rats in the BA model group showed symptoms of asthma such as sneezing, cough and gasping. The rats in the COPD model group were characterized by a thin and small body, withered hair and tachypnea. In contrast, the rats in the COPD+BA model group showed symptoms of both the COPD and BA models. Moreover, their general condition appeared to be rather worse.

The histopathology of BA is characterized by a number of structural changes, including hyperplasia of the mucus glands, subepithelial fibrosis, infiltration of inflammatory cells, and hypertrophy of bronchial smooth muscle, which can be observed in the proximal airways as well as in the distal lung.<sup>8</sup> COPD is a slowly-progressive chronic inflammatory condition that affects the conducting airways, both large and small, and the pulmonary parenchyma.<sup>9</sup> Our results were in concordance with this research. However, we noticed that the histopathology of the COPD+BA model group was similar to that of the COPD model group, differing only in the infiltration of eosinophils with diffuse lesions, which might be due to

the interaction of two different mechanisms.

Our results showed that the AHR of the BA model group was the highest while that of the COPD model group was the lowest of these three groups. We think these changes may be related to their different pathological features. AHR is thought to be a characteristic of BA,<sup>10</sup> while COPD rarely shows AHR. At the same time, two different inflammatory mechanisms might act concordantly in COPD+BA, which could orchestrate the interaction of different pathogenic cells, cytokines, inflammatory media, signaling pathways and the expression of genes. This interaction could lead to the pathophysiological features of either BA or COPD to be strengthened or weakened. These changes would be worthy of further investigation.

Many inflammatory cells and cytokines have important roles in the pathogenesis of BA and COPD. Previous investigations have shown that there is an increased number of CD4<sup>+</sup> T cells in the airways of asthmatic patients and that these are predominantly T helper 2 (Th2) cells. In contrast to asthma, the CD4<sup>+</sup> T cells that accumulate in the airways and lungs of patients with COPD are mainly Th1 cells.<sup>11</sup>

We know that IL-4, IL-5 and IFN- $\gamma$  are lymphokines. IL-4 is also a growth factor for B cells. In addition to mediating T cells growth, it is a principal factor that controls the differential development of Th0 cells into Th1 and Th2 cells.<sup>12</sup> IL-5, which is produced by mast cells, is also a Th2-type cytokine. It can induce the synthesis of IgA and promote the proliferation and differentiation of eosinophils.<sup>13</sup> IFN- $\gamma$  is a Th1-type cytokine that generally counteracts the Th2 response. The delivery of IFN- $\gamma$  could be an effective strategy for regulating Ag-induced immune responses in the lung.<sup>14</sup> Our results showed that the serum IL-4 concentration in the COPD model group was lower than that in the control group, while that in the BA model group had the tendency to be higher, which is in agreement with prior studies.<sup>11</sup> However, the serum IL-4 concentration in the COPD+BA model group was higher than that in the COPD model group, which might show that IL-4 is likely to be involved in the inflammatory process of COPD+BA. Alternatively, it could be a strengthening of the BA response. We also found that the serum IL-5 concentration in the COPD model group was significantly higher than that of the other three groups, which is again in agreement with prior investigations.<sup>15</sup> The serum IL-5 concentration in the BA model group did not increase, while the serum IFN- $\gamma$  concentration in the COPD model group also remained low. These phenomena might indicate that there is another immunological mechanism involved in the pathogenesis of BA and COPD. In our study, the serum IFN- $\gamma$  concentration in the COPD+BA model group was decreased compared to that of the other groups. We could presume that Th2 cytokines are preferentially expressed in the inflammatory process of

COPD+BA, which might demonstrate an imbalance of IFN- $\gamma$ /IL-4.

Both IL-6 and TNF- $\alpha$  are proinflammatory cytokines. Differing from prior researches,<sup>5,16</sup> our results showed that the serum IL-6 concentration in the COPD model group decreased while that in the BA model group did not change compared to that in the control group. This might be associated with experimental error or bias. However, there was no significant difference in the serum IL-6 concentration between the COPD and COPD+BA model groups, which might show that serum IL-6 is more closely associated with BA and the IL-6 expression in the COPD+BA model group was inhibited. Some researchers have argued that TNF- $\alpha$  is a strong candidate for a mediator that drives blood vessel remodeling and lymphangiogenesis in inflammation.<sup>17</sup> Our study showed that the serum TNF- $\alpha$  concentrations of both the COPD and BA model groups were higher than that of the control group, while that of the COPD+BA model group were lower. This phenomenon also needs further investigation.

Evidence has suggested that IL-8 is released in vivo by inflammatory cells in asthma,<sup>18</sup> which agrees with our study's results. However, we found that the serum IL-8 concentration of the COPD+BA model group was the highest in all groups, which might indicate that IL-8 is involved in the inflammatory progress of both BA and COPD+BA. It could also indicate that the IL-8 expression of the COPD+BA group has been increased.

As mentioned above, we found that there is a prominent expression of Th2 cells in the BA model group, while the COPD model group has mainly Th1 cells. However, the expression of inflammatory cytokines in the COPD+BA model group might be up or downregulated when both disease processes are present. COPD+BA deserves further investigation.

Recent studies have shown that the stress response and dysregulation of cytokines can trigger the HPA axis and the sympathetic nervous system, and subsequently, asthmatic patients have an imbalance of their Neuro-Endocrine-Immunity (NEI) network.<sup>19</sup> Our previous studies have suggested that some herbs, such as epimedium, have a regulatory effect on the disorder of the NEI.<sup>20,21</sup>

A growing number of studies have recognized that asthmatic patients may have an attenuated activity and/or responsiveness of their HPA axis,<sup>2</sup> although some studies suggest that the HPA axis function in adult asthmatic patients has not been suppressed.<sup>3</sup> Our previous studies show that asthmatic rats display a multiple-level disorder of HPA axis.<sup>22</sup> These differing conclusions deserve further investigation. Meanwhile, some researchers have found that patients with COPD who are on inhalant glucocorticoid treatment had a lower risk of adrenal cortical insufficiency than those on oral glucocorticoid

treatment. But there seem to be no clinical significance of these findings.<sup>4</sup> However, we know little about the function of the HPA axis in patients with COPD+BA. Additional well-designed research is required before any definite conclusions can be reached.

In our study, we compared the levels of CRH mRNA, plasma ACTH and serum CORT in these three model groups. The results showed that the levels of CRH mRNA and serum CORT in the BA model group increased compared to those in the control group, which might be due to the stress response of asthma attack or the disturbance of the HPA axis function. However, the CRH mRNA levels in the COPD and COPD+BA model group, especially in the COPD+BA model group, were both significantly lower than that in the control group. This phenomena might indicate that the HPA axis functions of both the COPD and COPD+BA model groups are impaired, and the fact that the COPD+BA model group had a greater attenuation may have resulted from the interaction of two different mechanisms. We also found that the serum CORT levels in both the COPD and COPD+BA model groups remarkably lower than that in the control group, which indicates that the HPA axis functions of these two groups might be impaired. However, the serum CORT level in the COPD+BA model group was higher than that in the COPD model group. This is probably associated with the stress reaction of asthmatic attack based on the attenuation of the HPA axis function in COPD. There was no significant difference among these four groups in plasma ACTH, which might imply that ACTH was relatively immune to the HPA axis dysfunction. From our results, we determine that the dysfunction of the HPA axis represents the attenuation of endogenous anti-inflammatory effect and these changes could contribute to the development of these diseases.

In conclusion, both BA and COPD are the most common inflammatory airway diseases, but their mechanisms are different. These two different mechanisms can affect each other in patients with COPD+BA, which can lead to a more complicated clinical manifestation. We can use BN rats to establish some reliable animal models of BA, COPD and COPD+BA. Our research suggests that the features of inflammation in rats in the COPD+BA model group seem to be exaggerated. The HPA axis functions in rats in these three model groups, especially in the COPD+BA model group, have been disturbed or impaired, which is prominent at the hypothalamic level. Further studies on the inflammatory features and HPA axis function of inflammatory diseases are necessary, and the increased knowledge would contribute to the treatment of these diseases.

#### REFERENCES

1. Laghi F, Adiguzel N, Tobin MJ. Endocrinological derangements in COPD. *Eur Respir J* 2009; 34: 975-996.
2. Priftis KN, Papadimitriou A, Nicolaidou P, Chrousos GP. The

- hypothalamic-pituitary-adrenal axis in asthmatic children. *Trends Endocrinol Metab* 2008; 19: 32-38.
3. Ringdal N, Lundback B, Alton M, Rak S, Eivindson A, Bratten G, et al. Comparable effects of inhaled fluticasone propionate and budesonide on the HPA-axis in adult asthmatic patients. *Respir Med* 2000; 94: 482-489.
  4. Santen RT, Schlaghecke R, Schwalen A. Hypothalamic-hypophyseal-adrenal axis suppression in patients with chronic obstructive pulmonary disease. A comparison of inhalant with systemic glucocorticoid administration. *Dtsch Med Wochenschr* 1997; 122: 497-503.
  5. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008; 118: 3546-3556.
  6. Hoshino T, Toda R, Aizawa H. Pharmacological treatment in asthma and COPD. *Allergol Int* 2009; 58: 341-346.
  7. Zheng H, Liu Y, Huang T, Fang Z, Li G, He S. Development and characterization of a rat model of chronic obstructive pulmonary disease (COPD) induced by sidestream cigarette smoke. *Toxicol Lett* 2009; 189: 225-234.
  8. Hamid Q. Gross pathology and histopathology of asthma. *J Allergy Clin Immunol* 2003; 111: 431-432.
  9. Molfino NA, Jeffery PK. Chronic obstructive pulmonary disease: histopathology, inflammation and potential therapies. *Pulm Pharmacol Ther* 2007; 20: 462-472.
  10. Berend N, Salome CM, King GG. Mechanisms of airway hyperresponsiveness in asthma. *Respirology* 2008; 13: 624-631.
  11. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008; 8: 183-192.
  12. Abehsira-Amar O, Gibert M, Jolij M, Theze J, Jankovic DL. IL-4 plays a dominant role in the differential development of Th0 into Th1 and Th2 cells. *J Immunol* 1992; 148: 3820-3829.
  13. Matthaei KI, Foster PS, Young IG. The role of interleukin-5 (IL-5) *in vivo*: studies with IL-5 deficient mice. *Mem Inst Oswaldo Cruz* 1997; 92: 63-68.
  14. Nakagome K, Okunishi K, Imamura M, Harad H, Matsumoto T, Tanaka R, et al. IFN-gamma attenuates antigen-induced overall immune response in the airway as a Th1-type immune regulatory cytokine. *J Immunol* 2009; 183: 209-220.
  15. Jiang WZ, Liu HZ. COPD exacerbation and Eosinophilia in airway. *J Mod Clin Med Bioeng* 2004; 10: 76-78.
  16. Karadag F, Karul AB, Cildag O, Yilmaz M, Ozcan H. Biomarkers of systemic inflammation in stable and exacerbation phases of COPD. *Lung* 2008; 186: 403-409.
  17. Baluk P, Yao LC, Feng J, Romano T, Jung SS, Schreiter JL. TNF-alpha drives remodeling of blood vessels and lymphatics in sustained airway inflammation in mice. *J Clin Invest* 2009; 119: 2954-2964.
  18. Yousefi S, Hemmann S, Weber M, Holzer C, Hartung K, Blaser K, et al. IL-8 is expressed by human peripheral-blood eosinophils-evidence for increased secretion in asthma. *J Immunol* 1995; 154: 5481-5490.
  19. Frieri M. Neuroimmunology and inflammation: implications for therapy of allergic and autoimmune diseases. *Ann Allergy Asthma Immunol* 2003; 90: 34-40.
  20. Shen ZY, Dong JC, Cai DF, Cai DP, Huang JH, Zhang XM. Important action of improving Adrenocortical function for certain diseases recovery. *Chin J Integr Tradit West Med (Chin)* 2007; 27: 364-367.
  21. Dong JC, Zhao FD, Xie JY. Effects of milkvetch root on Neuro-endocrino-immune network in asthma model rat. *Chin J Integr Tradit West Med (Chin)* 2007; 27: 619-622.
  22. Gong ZH, Dong JC, Xie JY, Liu BJ, Duan XH, Zhao FD, et al. Regulatory function of Shen-reinforcing and Qi-supplementing drugs on Hypothalamic-pituitary-adrenal axis and interleukin-6 dysfunction in asthmatic rats. *Chin J Integr Tradit West Med (Chin)* 2008; 28: 348-351.

(Received November 30, 2009)

Edited by WANG De