

A Rat Model for Stable Chronic Obstructive Pulmonary Disease Induced by Cigarette Smoke Inhalation and Repetitive Bacterial Infection

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To develop a stable chronic obstructive pulmonary disease (COPD) model in rats. Sprague-Dawley rats were treated with cigarette-smoke inhalation (CSI) for 12 weeks, repetitive bacterial infection (RBI) for 8 weeks, or the combination of the two (CCR) for 12 weeks and followed up for the additional 20 weeks. Tidal volume (V_T), peak expiratory flow (PEF) and 50% V_T expiratory flow (EF_{50}), histological changes in the lungs, and levels of the cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-8, and IL-10 in serum and bronchial alveolar lavage fluid (BALF) were examined at intervals during the 32 week study period. The right ventricular hypertrophy index (RVHI) was also determined at the same times. V_T , PEF, and EF_{50} were decreased in rats with COPD compared to the control. The expression of TNF- α , IL-8 and IL-10 increased in both serum and BALF with a similar trend. Bronchiole and arteriole wall thickness and the degree of bronchiole stenosis and alveolar size increased in COPD rats. RVHI was reduced gradually following the treatment. All of these changes were more pronounced in the CCR-treatment group than in the other groups. Our results have shown that CSI or RBI alone can induce COPD in rats, but that the combination of CSI with RBI induces a stable COPD that has more similarity to complications seen in patients with COPD. This combination may therefore provide a more appropriate model for study of human COPD.

Key words pulmonary disease; chronic obstructive; animal model; pulmonary function; histological change; right ventricular hypertrophy index

Chronic obstructive pulmonary disease (COPD) has been defined as a preventable and treatable pathologic condition characterized by partially reversible airflow limitation,¹ and is a major cause of morbidity and mortality throughout the world.² Its prevalence among the Chinese population aged 40 or older is 8.2%.³ Development of COPD is slow and progressive in humans, with occasional exacerbations caused by an inflammatory response to triggering substances such as noxious gases,¹ bacteria^{4–6} or viruses.^{7–11} Four abnormalities are present in chronic, stable COPD: emphysema, small airway remodeling, pulmonary hypertension, and chronic bronchitis.

Tobacco smoking and bacterial infection are the most common and important risk factors for COPD,^{1,12,13} and they have each been used to establish animal COPD models.^{14–16} Animal models have also used other noxious gases^{17–19} and *Pneumocystis carinii* infection²⁰ in COPD induction. Short term induction protocols (days)^{17,21} have resulted in a pulmonary inflammatory infiltrate, increased mucus production, and pulmonary edema. Long term induction protocols (weeks or months)^{15,17–19} have produced, in addition to the inflammatory infiltrate, emphysema and pulmonary remodeling characterized by fibrosis, and thickened bronchiole and arterial walls. Problems with animal models are that most of them are of short duration and the COPD produced does not correspond to the late chronic disease stage that is responsible for the morbidity and mortality in humans. Another problem is that the disease produced by cigarette smoke in animals is mild, and probably similar to the early GOLD stage 1 and 2 of COPD in humans.

The occurrence of exacerbations, which are very often

caused by bacterial or viral infections, increases the severity of COPD and causes a higher death rate in humans.²² Bacterial infection is a common cause of exacerbations in the development of COPD. The proportion of Gram negative bacilli, such as *Klebsiella pneumoniae*, infections is increasing among lung infections, especially in the elderly. *Klebsiella pneumoniae* is one of top three pathogens causing COPD deterioration and community acquired pneumonia,^{4–6} and has been used to prepare animal models of COPD at the stable stage¹⁵ and the acute exacerbation stage.²³ Therefore, in the present study, *Klebsiella pneumoniae* was used in our rat COPD models to mimic the clinical pathology of human COPD. We hypothesized that combining cigarette-smoke and repetitive bacterial infection in a rat model of COPD would produce a disease stage with more similarity to late stage human disease than models using only one initiator. In this study, a new model combining cigarette smoke inhalation (CSI) and repetitive bacterial infection (RBI) was established and its features compared to models using CSI or RBI alone in order to try to establish a new, more clinically relevant COPD model in rats.

MATERIALS AND METHODS

Animals Eighty male and 80 female 8 week old Sprague-Dawley rats weighing (203.5±8.4)g (Laboratory Animal Center of Henan Province, China) were randomly divided into the following four groups (20 male and 20 female rats in each group): control, cigarette-smoke inhalation (smoke), repetitive bacterial infection (bacteria), and smoke+bacteria (smoke/bacteria) groups. Experimental protocols were approved by the Experimental Animal Care and Ethics Committees of the First Affiliated Hospital, Henan University of

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Traditional Chinese Medicine. All rats arrived at the animal facility of the First Affiliated Hospital 7 d before the experiment, were housed under standardized environmental conditions, and given food and water *ad libitum*.

Klebsiella pneumoniae *Klebsiella pneumoniae* (strain ID: 46114) was purchased from National Center For Medical Culture Collection of National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and diluted in normal saline at a concentration of 6×10^8 colony forming units (CFU) per milliliter before administering to animals. The concentration of bacteria in the dilution was confirmed in pilot experiments.

Cigarettes Hongqi Canal[®] Filter tip cigarette (tobacco type, tar: 14 mg, nicotine content: 1.2 mg, carbon monoxide: 15 mg, Anyang Cigarette Factory, Henan Province).

COPD Models *Protocol for Cigarette Smoke Inhalation:* Rats were exposed to tobacco smoke of 8 cigarettes per treatment twice a day during the first two weeks, and to tobacco smoke of 15 cigarettes per treatment, three times a day, from the third to the twelfth week. The rats were placed inside a closed box connected to the smoke source, and received two or three 30-min exposures per day with a three-hour interval between them.^{16,24–26}

Protocol for Repetitive Bacterial Infection: A *Klebsiella pneumoniae* dilution (6×10^8 CFU/mL, 0.1 mL) was slowly dropped in an alternate fashion into the rat's two nostrils once a day every 5 d for 8 weeks.¹⁵

Protocol for Cigarette Smoke+Repetitive Bacterial Infection: Rats were treated with a combination of the cigarette smoke inhalation and repetitive bacterial infection (CCR) protocols described above.

Pulmonary Function Measurements Pulmonary function was measured in weeks 0, 8, 12, 16, 20, 24 and 32 after the initiation of treatment. To measure pulmonary function, rats were placed in a sealed unrestrained Whole Body Plethysmograph (UWBP, Buxco Electronics, Troy, NY, U.S.A.) connected to a transducer and computer. As the animal breathes in and out, the up and down movement of the thorax cage changes the volume of the box. These changes in volume are then converted to electrical signals through a pressure transducer and amplifier, and processed by computer. The respiratory curve is displayed on a computer screen, the graphics analyzed by the software, and finally tidal volume (V_T), peak expiratory flow (PEF) and 50% tidal volume expiratory flow (EF_{50}) calculated.

Inflammatory Cytokines in Bronchoalveolar Lavage Fluid and in Serum In weeks 8, 12, 16, 20, 24 and 32, rats were sacrificed, and levels of the cytokines TNF- α , IL-8 and IL-10 measured in serum and bronchoalveolar lavage fluid (BALF), using enzyme-linked immunosorbent assay (ELISA) kits (Boster Bio-Engineering Co., Ltd., Wuhan, China).

After exteriorization of the left lung, BALF was obtained by irrigating it three times with 3 mL of cold normal saline through a tracheal cannula, and finally collecting this fluid in a tube through a gauze filter. The coefficient of recovery was more than 80%. BALF was centrifuged at 2000 rpm for 10 min, and the supernatant collected for cytokine measurements.

Histological Changes in Lung, Bronchus and Arteriole In weeks 8, 12, 16, 20, 24 and 32 after treatment, lung tissues from the sacrificed animals were removed under normal

atmosphere and cut into slices along the maximum diameter of the right lower lobe, with a thickness of 3 mm, and fixed in 4% paraformaldehyde solution for 72 h. The samples were then embedded in paraffin, sliced into 4-micron sections. The sections were stained with hematoxylin-eosin stain, and photographed. All images were taken using an Olympus PM-10AD optical microscope and photographic system (Olympus Optical Co., Ltd., Japan).

Determination of Bronchiole and Pulmonary Arteriole Wall Thickness: For wall thickness measurements, images were taken at a magnification of $\times 200$ and analyzed with the Image-Pro[®] Plus 6.0 professional image analysis system (Media Cybernetics, Inc., U.S.A.). For each section, the microscopy field was divided into 9 regions. Five of the 9 regions were then selected randomly, and the bronchiole and pulmonary arteries were examined. The bronchiole or pulmonary arterial wall thickness in each rat was determined from averaged data of 5 measurements from one section.

Assessment of Bronchiole Stenosis: The normal bronchiole lumen area was set to 1. Bronchiole stenosis was then graded on a scale of 0 to 3 according to the fraction of the normal area that was lost: Level 0: normal; Level 1: less than a quarter ($< 1/4$); Level 2: a quarter to a half ($1/4$ to $1/2$); Level 3: more than a half ($> 1/2$).

Evaluation of Alveolar Number and Size: Under microscopy ($\times 100$), 5 photographs were taken in each slice, the alveolar number in a quarter of the area was counted and the diameters measured with Image-Pro[®] Plus 6.0 software.

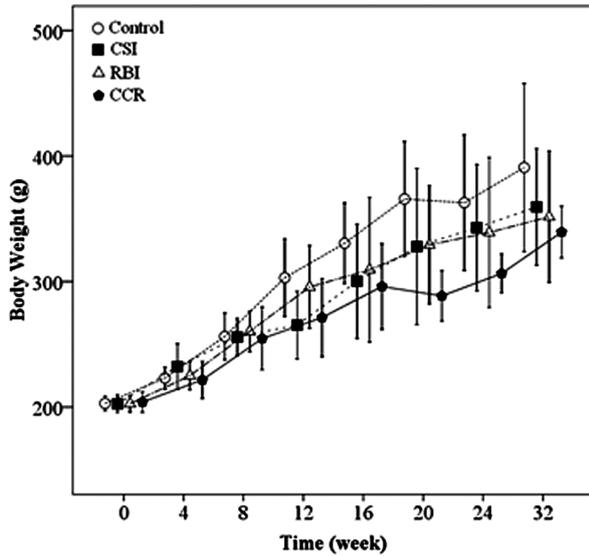
Right Ventricular Hypertrophy Index (RVHI) After removing the arterial and adipose tissue on the epicardium, the right ventricle (RV), left ventricle (LV) and interventricular septum (S) were separated and weighed. The RVHI was calculated through an equation: $RVHI = RV / (LV + S)$.²⁷

Statistical Analysis Data from experimental measurements were expressed as mean \pm S.D. For repeatedly measured data (body weight, tidal volume, peak expiratory flow, and 50% tidal volume expiratory flow), generalized estimating equations (GEE) were applied to detect the difference in overall group effect as well as the linear trend over time of each experimental group compared to control group, after considering the correlation of data within the same rats. For serum and histological data from sacrificed rats, general linear models were applied to detect the difference in overall group effect as well as the linear trend over time of each experimental group compared to control group. The adjusted mean difference, which indicates the average difference of experimental group compared to control group after controlling for time and gender effects, was calculated. Statistical analyses were performed with SAS software version 9.2 (SAS Institute Inc., Cary, NC, U.S.A.). A two-tailed $p < 0.05$ indicated statistical significance.

RESULTS

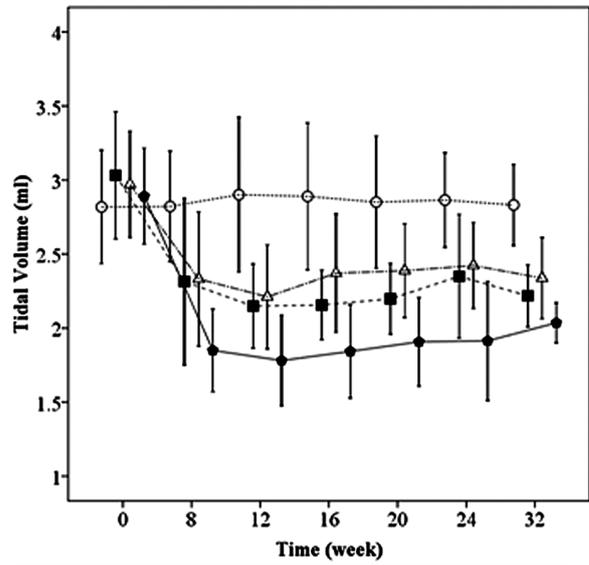
Animal Condition Mortality: During the eight-month-period, two rats died in the CSI-treated group, two in RBI-treated group, and three in CCR-treated group (Table 1). Five rats died due to pulmonary abscesses, and two died due to foot and ear abscesses after fighting with other rats. One control rat died during the pulmonary function test procedure because of an operational error. In each group, there were more

A



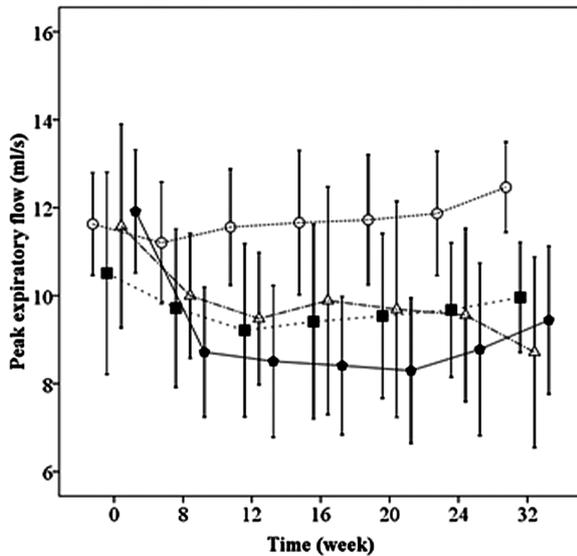
Group	0	4	8	12	16	20	24	32
CCR	19	19	13	14	10	10	11	7
CSI	20	20	18	12	10	9	12	9
RBI	20	20	15	9	10	10	8	8
Control	20	20	13	9	10	9	10	6

B



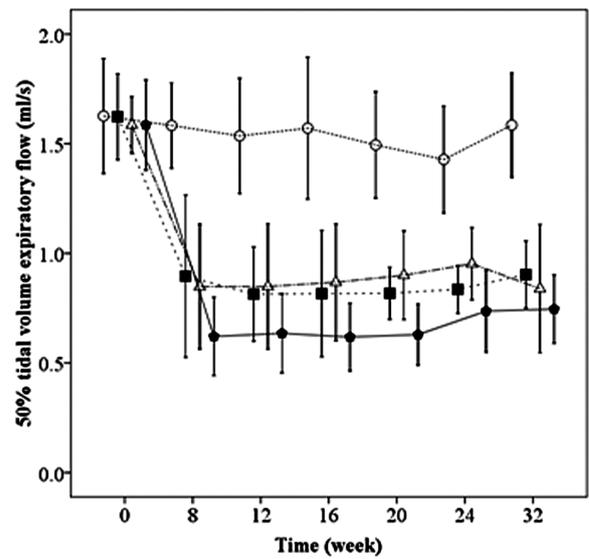
Group	0	8	12	16	20	24	32
CCR	19	13	14	10	10	11	7
CSI	20	18	12	10	9	12	9
RBI	20	15	9	10	10	8	8
Control	20	13	9	10	9	10	6

C



Group	0	8	12	16	20	24	32
CCR	19	13	14	10	10	11	7
CSI	20	18	12	10	9	12	9
RBI	20	15	9	10	10	8	8
Control	20	13	9	10	9	10	6

D



Group	0	8	12	16	20	24	32
CCR	19	13	14	10	10	11	7
CSI	20	18	12	10	9	12	9
RBI	20	15	9	10	10	8	8
Control	20	13	9	10	9	10	6

Fig. 1. Temporal Changes in Body Weight (A), Tidal Volume (B), Peak Expiratory Flow (C), and 50% Tidal Volume Expiratory Flow (D) over a Period of 32 Weeks in Rats of the Control, CSI, RBI, or CCR Groups

Values are mean±S.D. Sample size in each group for each time point was listed below the figure. A slight separation of error-bars among groups within the same time point was made to avoid overlapping.

than 6 animals qualified for analysis.

Body Weight: The 3 COPD groups gained weight at a slower rate than control (Fig. 1A), as shown by their linear trends in body weight with time (CCR vs. control $p<0.0001$, CSI vs. control $p=0.0171$, RBI vs. control $p=0.0205$). Weight gain was slowest in the CCR group and the linear trend for this group was significantly slower than that of the CSI ($p=0.0418$) or

RBI ($p=0.0307$) groups. No difference was seen between CSI and RBI groups.

Pulmonary Function Pulmonary function worsened with time in all 3 COPD groups, but did so to a greater extent in the CCR group. Tidal volume declined about 25–33% after 8 weeks of treatment in all COPD groups (Fig. 1B), and the linear trends with time of these groups for this parameter

Table 1. Mortality of Rats in 4 Different Groups

Group	No. of rats	No. of death	Mortality
Control	40	1	2.5%
CSI	40	2	5.0%
RBI	40	2	5.0%
CCR	40	3	7.5%

were significantly different from control (CCR $p<0.0001$, CSI $p<0.0001$, RBI $p=0.0005$), but not from each other. The CCR group, after adjusting for time and gender, had a significantly lower mean tidal volume than the other 3 groups (adjusted mean difference: CCR vs. RBI, -0.2237 mL, $p=0.0195$; CCR vs. CSI, -0.2262 mL, $p=0.0385$; CCR vs. control, -0.2793 mL, $p=0.0074$).

Peak expiratory flow also decreased with time in all COPD groups (Fig. 1C) but not in control. The linear trend over time reached statistical significance compared to control in the CCR and RBI groups (linear trend over time: $p<0.0001$ and $p=0.0002$, respectively). In addition, a significant difference in linear trend was found between the CCR and CSI groups ($p=0.0153$). However, no significant difference in linear trend was seen between the RBI and CSI groups ($p=0.7148$). Moreover, the adjusted mean difference indicated that only CSI had a significantly lower mean peak expiratory flow than the RBI and control groups (CSI vs. RBI, -1.1164 mL/s, $p=0.0442$; CSI vs. control, -1.316 mL/s, $p=0.0044$).

As seen with tidal volume and peak expiratory flow, 50% tidal volume expiratory flow (Fig. 1D) decreased with time in all 3 COPD groups (linear trend over time vs. control, all $p<0.0001$). In addition, all COPD groups had significantly lower mean values for this parameter than to control (adjusted mean difference: CCR vs. control, -0.4187 mL/s, $p<0.0001$; CSI vs. control, -0.3057 mL/s, $p<0.0001$; RBI vs. control, -0.3022 mL/s, $p<0.0001$). However, no difference was found between the 3 COPD groups in either adjusted mean or linear trend.

Inflammatory Cytokines All COPD groups had higher levels of TNF- α , IL-8, and IL-10 than control in both serum and BALF (Fig. 2). Cytokine levels decreased somewhat with time in all experimental groups, but these levels were higher in the CCR group than in the other 2 COPD groups at all times. The CCR group also had significantly higher mean levels than the other COPD groups of all cytokines in both serum and BALF (all p -value <0.001).

For serum TNF- α (Fig. 2A), all treatment groups had linear trends with time that were significantly different from the control group (all $p<0.0001$) and significantly higher means compared to control (adjusted mean difference: CCR vs. control, 331 ng/L, $p<0.0001$; CSI vs. control, 197 ng/L, $p<0.0001$; RBI vs. control, 246 ng/L, $p<0.0001$). The results for other cytokines in serum and BALF (Fig. 2, Panels B to F) were similar to those for serum TNF- α . The overall group effects and linear trends over time of the 3 experimental groups were significantly different from control (all $p<0.0001$). In addition, the adjusted mean difference also showed CCR to have a significantly higher mean cytokine level than CSI and RBI in either serum or BALF (all p -value <0.001).

Airway Remodeling Bronchiole wall thickness increased with time in all treated groups, and this increase was greatest

in the CCR group (Fig. 3A). The CCR and RBI groups had significantly higher mean bronchiole wall thickness than both control (adjusted mean difference: CCR vs. control, 61.85 pixels, $p<0.0001$; RBI vs. control, 43.61 pixels, $p<0.0001$) and CSI groups (adjusted mean difference: CCR vs. CSI, 54.01 pixels, $p<0.0001$; RBI vs. CSI, 35.77 pixels, $p=0.0002$). No difference in this parameter was found between CCR and RBI, and linear trends over time of 3 experimental groups were not significantly different from control.

Airway Obstruction Bronchiole stenosis increased with time in all treated groups, and this increase was greatest in the CCR group (Fig. 3B). After controlling for the time effect, CCR, CSI, and RBI had significantly higher mean bronchiole stenosis scores than control (adjusted mean difference: CCR vs. control, 2.42 , $p<0.0001$; CSI vs. control, 1.43 , $p<0.0001$; RBI vs. control, 2.50 , $p<0.0001$). In addition the CCR and RBI groups had significantly higher mean scores of bronchiole stenosis than CSI (adjusted mean difference: CCR vs. CSI, 0.983 , $p=0.0103$; RBI vs. CSI, 1.067 , $p=0.0055$). In addition, the linear trend over time of the RBI group was significantly different from that of control ($p=0.0018$).

Emphysema Alveolar number decreased and alveolar diameter increased with time, again with the most pronounced effect in the CCR group (Figs. 3C and D). Alveolar number was lower than control in the CCR group, but not the other two COPD groups (adjusted mean difference: CCR vs. control, -13.42 , $p<0.0001$). The CCR group also had a significantly lower mean alveolar number than the CSI and RBI groups (adjusted mean difference: CCR vs. CSI, -8.235 , $p=0.0025$; CCR vs. RBI, -9.897 , $p=0.0003$). The linear trends over time of the 3 experimental groups were not significantly different from that of control, although the linear trend of the CCR group was significantly different from that of the RBI group ($p=0.0459$).

Alveolar diameters in COPD experimental groups were significantly greater than control (adjusted mean difference: CCR vs. control, 235 pixels, $p<0.0001$; CSI vs. control, 233 pixels, $p<0.0001$; RBI vs. control, 170 pixels, $p<0.0001$) but were not significantly different from each other. The linear trends over time of the COPD groups were not significantly different from that of control. However, the linear trends of CCR and RBI were significantly different from that of CSI ($p=0.0027$ and $p=0.0289$, respectively).

Pulmonary Hypertension Evidence of pulmonary hypertension was evaluated by measuring arteriole wall thickness and RVHI. Arteriole wall thickness was increased in COPD rats, but RVHI, surprisingly, was decreased (Fig. 3E). All three COPD groups had significantly higher mean alveolar wall thickness than control (adjusted mean difference: CCR vs. control, 52.64 pixels, $p<0.0001$; CSI vs. control, 17.77 pixels, $p=0.0009$; RBI vs. control, 42.02 pixels, $p<0.0001$). The CCR group also had a significantly higher mean alveolar wall thickness than the other 2 COPD groups (adjusted mean difference: CCR vs. CSI, 34.87 pixels, $p<0.0001$; CCR vs. RBI, 10.62 pixels, $p=0.044$). The linear trends over time of the 3 experimental groups were not significantly different from that of control. However, the linear trend was significantly different between RBI and CSI ($p=0.0101$).

Mean RVHI was significantly lower in all 3 experimental groups than in control (Fig. 3F; adjusted mean difference: CCR vs. control, -0.12 , $p<0.0001$; CSI vs. control, -0.07 ,

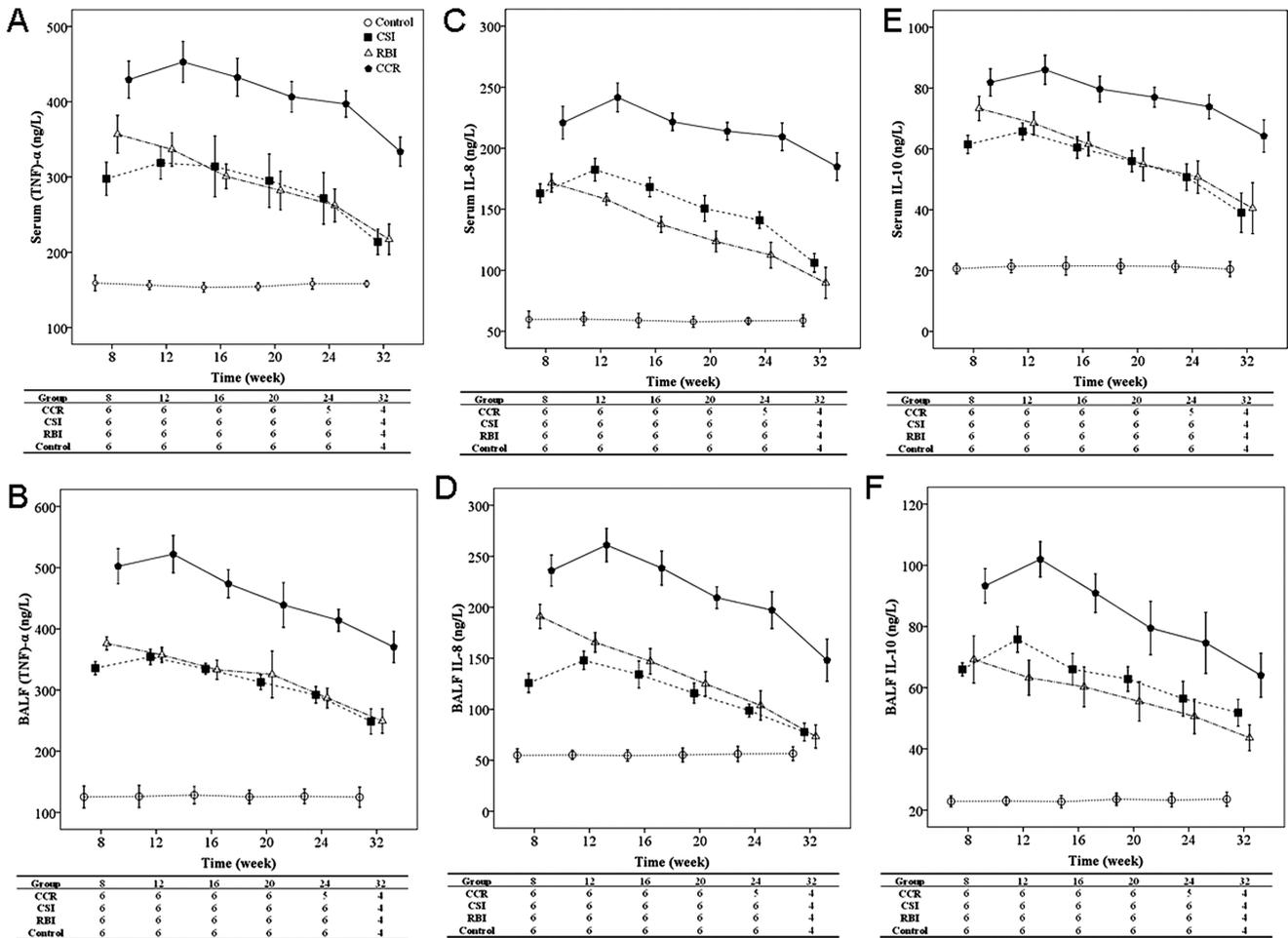


Fig. 2. Temporal Changes in TNF-α (A, B), IL-8 (C, D) and IL-10 (E, F) in Blood Serum (A, C, E) and Bronchoalveolar Lavage Fluid (B, D, F) over a Period of 32 Weeks in Rats of the Control, CSI, RBI, or CCR Groups

Values are mean±S.D. Sample size in each group for each time point was listed below the figure. A slight separation of error-bars among groups within the same time point was made to avoid overlapping.

$p=0.0077$; RBI vs. control, -0.11 , $p<0.0001$). No differences were seen among 3 experimental groups. The linear trend over time of RBI was significantly different from that of control ($p=0.0171$).

Lung Morphology As shown in Fig. 4, clearly visible bronchiole stenosis, pulmonary bronchiole expansion, alveolar destruction, and pulmonary small artery endothelial cell hypertrophy thickening could be seen in CSI-, RBI- and CCR-treated rats after 8 weeks of treatment, with the greatest effect seen in CCR-treated rats. These abnormalities became more severe with time, especially in CCR-treated rats, who had multiple lesions, such as airway inflammation, emphysema, small airway and arteriolar remodeling. No damage was observed in lung tissues of the control rats.

DISCUSSION

This is the first study to compare rat models of COPD using cigarette smoke exposure, repetitive bacterial infection, and the combination of the two protocols in order to find the most appropriate model for mimicking human COPD. All rats in each model exhibited COPD-like pathophysiological pulmonary features; however, the CCR protocol produced more severe COPD than the cigarette smoke or bacterial infection

models.

The poor weight gain in the three rat COPD models is related to the occurrence and development of airflow obstruction.²⁸⁾ Numerous studies have shown that COPD patients develop skeletal muscle atrophy and body weight loss, and the non-fat weight loss may influence the function of respiratory muscles and peripheral muscles, motor function, health state and prognosis of these patients.²⁹⁻³¹⁾ Reduction in body mass index (BMI) is an indicator of deteriorated nutrition, and has been found to increase the hospitalization rate in patients with COPD,³²⁾ elevate the rate of mechanical ventilation necessary, and increase mortality.³³⁾ In addition, the survival time of patients with COPD deterioration is highly related to the BMI. The reduction of motor function in COPD patients is associated not only with lung function but also with the dysfunction and atrophy of skeletal muscle.³⁴⁾

It is well known that cigarette smoke and bacterial infections are the most important risk factors for COPD, and that each of them can accelerate the development of COPD in humans. In this study, we found that CCR treatment took a shorter time to cause COPD than CSI or RBI treatment and that by the first 8 weeks; it produced a significant reduction in pulmonary function, chronic bronchitis, and small airway obstruction, pulmonary small airway and arteriolar remodeling

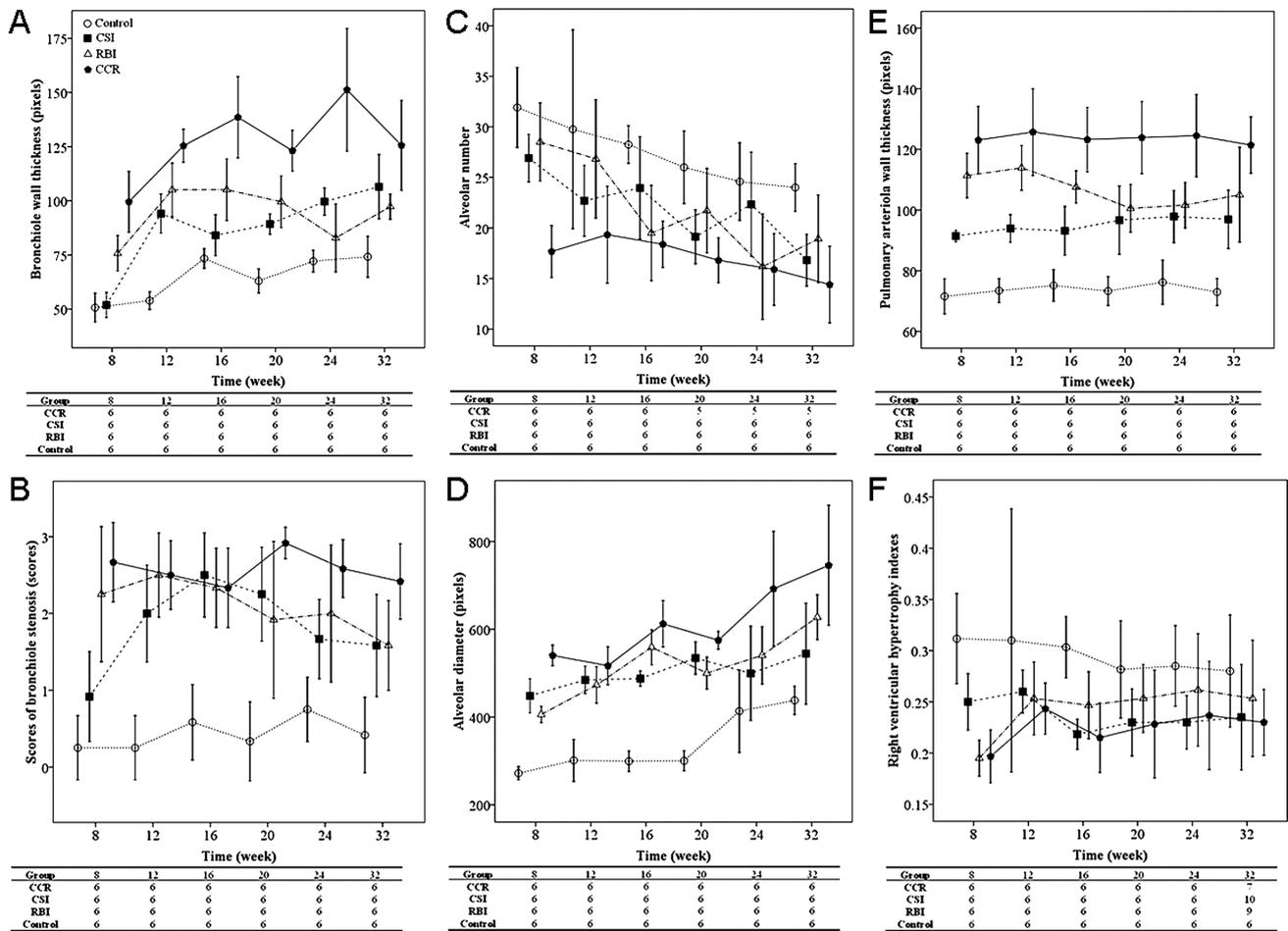


Fig. 3. Temporal Changes in Bronchiole Wall Thickness (A), the Scores of Bronchiole Stenosis (B), the Alveolar Number (C), Alveolar Diameter (D), Arteriolar Wall Thickness (E) and Right Ventricular Hypertrophy Indexes (F) over a Period of 32 Weeks in Rats of the Control, CSI, RBI, or CCR Groups

Values are mean±S.D. Sample size in each group for each time point was listed below the figure. A slight separation of error-bars among groups within the same time point was made to avoid overlapping.

and even extensive emphysema. After the first 8 weeks, rats exposed to cigarette smoke alone showed clear chronic bronchitis and local emphysema associated with small airway remodeling, and the rats exposed to recurrent bacterial infection alone showed significant inflammatory cell infiltration, airway mucosal edema, an increase in mucus secretion, localized emphysema, gas cavity stenosis, and other pathological manifestations. A major finding in the study was that the combination treatment causes changes in pulmonary function (such as decreases in V_T , PEF and EF_{50}) as well as clear emphysema, small airway remodeling and chronic bronchitis, and histological changes such as pulmonary and airway inflammation, enlargement of lung air space, pulmonary hyperinflation, and pulmonary hypertension. The impaired pulmonary function was consistent with the pathological changes in the lungs and was irreversible over the 32 week observation period.

RVHI decreased in all three treatment groups in our study, although in other COPD studies RVHI has increased. In our study COPD was induced in a short time, 8 weeks. The rapid increase in afterload in the right ventricle that would occur during this short induction period would produce thinning and decompensation of the right ventricle and a decrease in RVHI. Later the RVHI decrease would gradually disappear as the disease became chronic and the ventricle stabilized and was

able to compensate fully for the increased afterload. In a study by Li *et al.*²³⁾ the COPD model was established in 17 weeks, 9 weeks longer than in our study, and the RVHI had a tendency to increase. Thus the changes in RVHI are closely related to the time for establishment of COPD and the intensity of the stimulus used to establish the animal model.

The development of COPD in an animal is a very complex process.³⁵⁾ Although several methods^{14,36–39)} have been used to cause COPD or emphysema in animals, the complications seen in human COPD are frequently not found in these experimental COPD animal models or their long term consistency over time has not been determined. Therefore, these animal models are only used in short-term observation and are not suitable for long term research and for clarifying the mechanisms of COPD. The current findings suggest that the double treatment experimental COPD model might be a more useful pharmacological tool for developing therapeutic drugs for treating this disease.

Many irritants have been used to create COPD or emphysema in animal models.^{35,40)} In addition to smoking^{36,37,41,42)} and bacterial infection^{43,44)} exposure to particulate air pollution,^{38,45)} occupational dust, smoke^{27,46)} and bio-fuels for cooking⁴⁷⁾ are also risk factors for COPD. Previous experimental COPD or emphysema models have been developed in

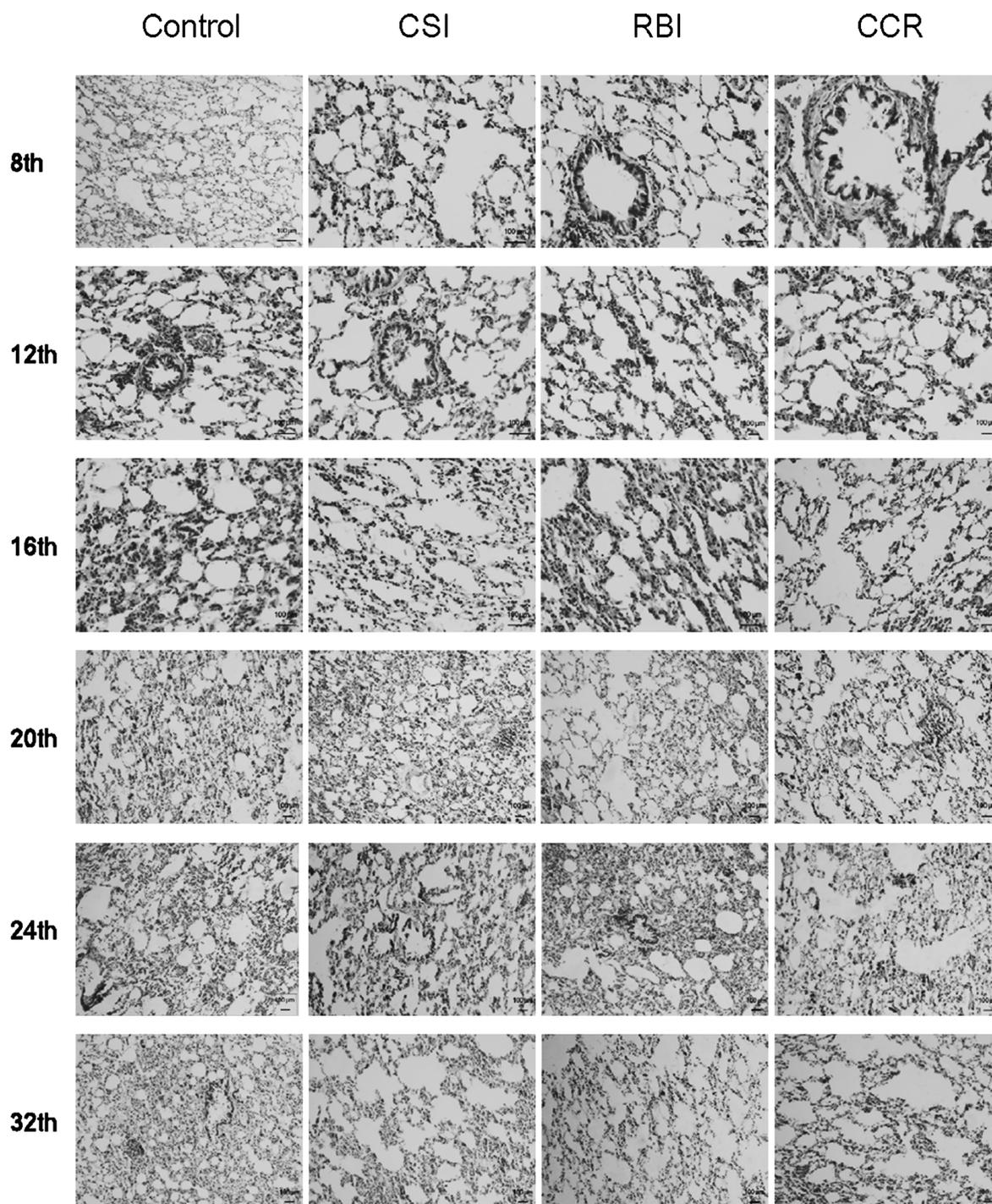


Fig. 4. Representative Pathological Changes in the Lungs, Stained by Hematoxylin-Eosin, in Rats of the Control, CSI, RBI, or CCR Groups

Alveolar and bronchiole morphological changes in the control, CSI-, RBI- and CCR-treated rats at the 8th week, the first row, magnification $\times 200$, at 12th week, the second row, magnification $\times 200$ (the first two panels) or $\times 100$ (the last two panels), at 16th week, the third row, magnification $\times 200$ (the first three panels) or $\times 100$ (the last panel), at 20th week, the fourth row, magnification $\times 100$, at 24th week, the fifth row, magnification $\times 100$, and at 32nd week, the sixth row, magnification $\times 100$.

guinea pigs,¹⁴⁾ mice,^{36,37)} rats,³⁸⁾ and dogs³⁹⁾ by exposing the animals to sulfur dioxide (SO₂),¹⁷⁾ smoke from solid fuel,¹⁹⁾ cigarette smoke,^{14,24,42)} lipopolysaccharide,³⁹⁾ proteases and their secretagogues, such as PAE, PPE, NE, proteinase 3, MMP,^{44,48,49)} and the factors used can enhance the effect of each other.^{50,51)}

However, the rat model seems to be more economical and more feasible, and smoke and bacterial infections appear to be the most frequently used initiators. Although all of the factors

used can induce COPD or emphysema-like pathophysiological pulmonary features, they are not similar to the disease occurring in patients with COPD. We, however, have developed a stable experimental COPD model in rats, using a combination of cigarette smoke inhalation and repeated *Klebsiella pneumoniae* infections, over a considerably shorter time period than animal models using cigarette smoke inhalation alone,⁵²⁾ providing more features of COPD and showing a longer consistency period. The COPD model developed in this study has

a similar profile to the processes that occur in COPD patients, and might be useful for elucidating the pathogenesis of COPD and be beneficial in the prevention and treatment of COPD.

COPD is a chronic disease significantly threatening the human health. It has a long disease course and often progresses within several decades in humans. Thus, to mimic the progression of human COPD in animals is very difficult. In previous COPD animal models, only pathology of COPD was observed in animals or the COPD in the animal model was observed for about 3 months, and long term observation of stable COPD was not carried out. Smoke exposure for 24 weeks to induce COPD was not reported in previous studies, and the stability of the COPD animal model was not evaluated after smoke exposure.⁵²⁾ Thus, to investigate the long term effect of COPD in these animal models is not suitable.

In the present study, our results showed that, at 8 weeks after smoke exposure, animals presented with pathological features of COPD and reduction of lung function, and our observation continued to 24 weeks after smoke exposure. That is, after establishment of COPD animal model, the animals were observed for about half a year, and results demonstrated the favorable stability of this COPD model. Thus, we speculate that this COPD animal model is suitable for the investigation of long term effect of COPD.

In conclusion, CCR can be used to prepare a rat COPD model in a short time period. Lung function is markedly compromised and the characteristic pathology of COPD (such as chronic bronchitis, emphysema, airway remodeling, pulmonary hypertension) is present. The COPD induced by CCR is more stable during a 32 week period than that induced by smoking or bacterial infection alone. In the present study, the CCR rat COPD model is more similar to the COPD seen in clinical practice than in other animal models, and is suitable for observation of both short and long term therapeutic efficacy and for studies on the pathogenesis of COPD.

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