ORIGINAL ARTICLE

Combined Effects of Chronic Obstructive Pulmonary Disease and Depression on Spatial Memory in Old Rats

Cui Cai^{1*}, Changqing Xu², Hualiang Jin³, Bei Li⁴

¹Department of Geriatrics, Hangzhou Red Cross Hospital, Hangzhou 310003, China

²Department of Respiratory, the Affiliated Hospital of Hangzhou Normal University, Hangzhou 330015, China, ³Department of Respiratory, ⁴Department of Geriatrics, Hangzhou First People's Hospital, Hangzhou 310006, China

Key words: chronic obstructive pulmonary disease; depression; spatial memory; aging; rats

Objective To investigate the combined effects of chronic obstructive pulmonary disease (COPD) and depression on spatial memory in old rats, aiming to better understand the comorbidity of the two diseases in geriatric patients.

Methods The SD rats were assigned into five groups: adult control group ($n=6$), elderly control group ($n=6$), elderly COPD group ($n=6$), elderly depression group ($n=6$) and elderly COPD with depression group ($n=6$). Smoking and chronic unpredictable mild stress (CUMS) with solitary support were used to induce COPD model, depression model, respectively, and the both were applied for the comorbidity model. Learning and memory deficits were assessed by Morris water maze (MWM) test. The activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in serum and hippocampus tissue were determined by Xanthinoxidase method and Thiobarbituric acid reaction (TBAR) method, respectively.

Results The results of pulmonary histology, lung function, open-field test and sucrose consumption demonstrated the comorbidity models of COPD and depression in elderly rats were successfully established using smoking and CUMS with solitary support. Compared with the elderly control group, the group of COPD with depression had obviously longer time of latency and longer travel distance to reach the platform in MWM test ($LSD-t=-10.116$, $P=0.000$; $LSD-t=-6.448$, $P=0.000$). The SOD activity in serum and hippocampus decreased significantly ($LSD-t=2.629$, $P=0.014$; $LSD-t=2.215$, $P=0.044$) and the MDA content in serum and hippocampus increased significantly ($LSD-t=-2.140$, $P=0.042$; $LSD-t=-2.070$, $P=0.049$) in elderly COPD with depression group.

Conclusions COPD in comorbidity of depression could induce spatial memory deficit in old rats. The

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*Corresponding author Tel: 86-0571-56109766; E-mail: caicui2003@aliyun.com

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mechanisms might be related to the overloaded and free radical metabolic imbalance. These results suggest a potential therapeutic target for comorbidity of COPD and depression in geriatric patients.

CHRONIC obstructive pulmonary disease (COPD) and depression are two of the most common diseases in elderly people. COPD is a preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation. It is due to the airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases. Researchers have found that the prevalence and the incidence of COPD increase with aging.¹⁻⁴ Depression is a common but a serious illness in elderly, especially in elder patients with COPD.^{5,6} COPD and depression always co-occur in elder patients, and the concurrence of both diseases among adults increases along with aging. With the aging of population worldwide, the prevalence of COPD and depression will increase in future. As a result, the relationship between COPD and depression in elderly people has clinically attracted more and more attention. However, the exact mechanism of these co-occurring phenomena in elderly patients is still unclear.

It has been well known that aging is associated with learning and memory deficits in both humans and animals. Spatial memory alteration is one of the first deficits observed in aging population. Chronic unpredictable mild stress (CUMS) is a method to establish a rat model of depression, which has been well accepted internationally. In the present study, the aging COPD rats were subjected to CUMS. The depression-like behavioral changes were observed in the Open-field test and Sucrose preference test. The effect of CUMS on spatial memory in old COPD rats were evaluated by using Morris water maze (MWM) test. The activity of superoxide dismutase (SOD) was detected by Xanthinoxidase method, and the content of malondialdehyde (MDA) was determined by Thiobarbituric acid reaction (TBAR) method. The primary objective of this study is to investigate the joint effects of COPD and depression on the spatial memory of old rats, which may provide a novel strategy in the treatment of the elderly patients with COPD and depression.

MATERIALS AND METHODS

Animals

The SD rats used in this study were purchased from

JOINN Laboratories (Suzhou, China). All animals received humane care and all experiments were performed according to the guidelines of the Committee for the Care and Use of Laboratory Animals of the hospital. Six healthy adult SD rats were in 4 months old, with mean weight of 200 ± 20 g, and 24 elderly SD rats were in 17 months old, half male and half female, with mean weights of 600 ± 50 g and 400 ± 40 g, respectively. Unless otherwise stated, the rats were kept under standard conditions (12 h light/dark cycle; lights on at 7:00 am; 22 ± 1 °C ambient temperature; $52 \pm 2\%$ relative humidity; with food and water *ad libitum*. After a 7-day adaptation to the standard conditions, the animals were assigned into five groups: adult control group ($n=6$), elderly control group ($n=6$), elderly COPD group ($n=6$), elderly depression group ($n=6$) and elderly COPD with depression group ($n=6$).

Establishments of animal models

COPD model

Rats were placed in a fumigating chamber, a special-glass box constructed by the laboratory. The volume of the chamber was 160 L. The rats were exposed to the smoke of 10 Chunghwa cigarettes (Shanghai Tobacco Group CO., LTD, Shanghai, China) for 30 minutes once a day, continued for 12 weeks in fumigating chamber.

Depression model

The rats were isolated from each other in a single cage and subjected to a variety of mild stressors: swimming in 4 °C cold water for 5 min, fasting for 24 h, water deprivation for 24 h, nipping tail for 1 min, wet bedding and cage tilting for 24 h, noising for 5 min, bundled for 6 h, or hang for 5 min. Rats received one of these stressors per day, but the same stressor was not applied in two consecutive days. The stress procedures were carried out for 3 weeks prior to the behavioral testing.

COPD with depression model

The rats were isolated with each rat in a single cage and subjected to smoking and a variety of mild stressors. The procedures of smoking and CUMS were consistent with the above described.

Measurement of lung function

Briefly, the rats were anaesthetized by an intraperito-

neal injection of 3% sodium pentobarbital (5 mg/kg) and maintained with an appropriate plane of the anesthesia. The trachea was opened with an inverted T-shaped incision between the 2nd and the 3rd cartilage ring, and then a T-type cannula was rapidly intubated. Then the rats were placed into an apparatus animal spirometer (Buxco, USA) for measuring pulmonary ventilation. One end of the cannula was connected to a pressure transducer which was applied to a pulmonary mechanic analyzer, and the other end was used for administration of air to expand the lungs.

To evaluate lung function of the experimental animal, we measured the ratio of forced expiratory volume at 0.2 s to the forced vital capacity (FEV_{0.2}/FVC) and the dynamic lung compliance (C_{ldyn}) with injecting 6.0 ml air through the T-typed cannula into the anaesthetized rats. Data were automatically recorded by the analyzer.

Histopathological study

The harvested lobes of lungs were fixed in 4% formaldehyde solution for 72 hours. The pulmonary tissues were then embedded in paraffin, cut into 4 µm thickness sections, stained with hematoxylin and eosin (H&E) solution, and applied onto a glass slide. Histology of lungs was observed and photographed using a light microscope (Olympus, Japan).

Open-field test

We used Open-field test to observe the depressive-like behavior in rats.⁷ The open-field device was made of opaque materials with square in shape and 80 cm×80 cm in size, where the bottom was divided equally into 25 squares, and surrounded by a wall in 40 cm high. The rat was put in the central square. The numbers of squares that the rat traversed in 3 minutes (only the squares on which the rat landed with four legs could be numbered as the score of horizontal activity) and the duration of standing on hind limbs were recorded. Each rat was measured for 3 minutes once. A score was given by each of two observers and the average value was taken.

Sucrose preference test

The animals were deprived of food and water for 24 h, and then served water and 1% sucrose solution for 1 h, followed by 48 h exposure to both water and sucrose solution. The positions of the two bottles (right/left) were varied randomly across animals and were

reversed after 30 min. The sucrose preference was calculated as: sucrose preference (%) = sucrose intake (g) / [sucrose intake (g) + water intake (g)] × 100%,⁸ which represented the mean values of daily tests over three days.

Morris water maze (MWM) test

Morris water maze test was used for evaluation of the spatial memory.⁹ A circular black pool (110 cm in diameter, 60 cm high, and 35 cm deep) was filled with water (23–24 °C). A circular platform (10 cm in diameter and 32 cm high) was placed within the pool in the center of the southwest quadrant and was submerged approximately 3 cm below the water surface. Outside the maze, fixed visual cues (i.e. a computer, hardware, and posters) were present at various locations around the room. Before each experiment, the rats were daily handled for 3 days and thus were habituated to the water maze for 30 s without a platform. The animals performed four trials each day for five consecutive days, and each trial began with rat being placed in the pool facing one of four side wall [labeled with North (N), East (E), South (S), and West (W), respectively] and released. The release positions were randomly predetermined for each trial by computer. The rats were allowed to swim until they found the platform and remained on it for 15 s. The time to reach the platform and the length of the swimming path were recorded by a video tracking system.

SOD and MDA in hippocampus and serum

SOD activity and MDA content were examined by chemical colorimetric analysis using multifunction enzyme marker (Infinite M200, Swiss Tecan, Switzerland). Total Superoxide Dismutase assay kit and Malondialdehyde assay kit (Jiancheng Bioengineering Institute, Nanjing, China) were used according to manufactures' instructions. Xanthinoxidase method was used to detect the activity of SOD, which was described by unit per milligram of hippocampus protein or per milliliter of serum, and one unit was defined as the amount of absorbance reduced by 50% at 550 nm. The content of MDA was determined by Thiobarbituric acid reaction (TBAR) method at wave length of 532 nm, and described by nmol per milligram of hippocampus protein or per milliliter of serum.

Statistical analysis

All data were analyzed using SPSS (version 17.0).

Data of horizontal movement were expressed as median and quantile, and Kruskal Wallis test was used to analyze the difference in the groups. Other data were described as mean $\pm$ SD, and the differences between the groups were analyzed using Analysis of Variance (ANOVA). LSD-*t* test was employed for multiple comparison among different groups. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Histopathologic analysis

Compared with the elderly control group, the deformed and damaged bronchial lumen were observed in the elderly COPD group and COPD with depression group, but not in the elderly depression group. There were epithelial cells and inflammatory exudate in the bronchial cavity, and the alveolar walls were thickened as a result of compensatory emphysema. lymphocytes, neutrophils and eosinophils were noted in these two groups (**Fig. 1**).

Pulmonary functions

The values of FEV_{0.2}/FVC and Cldyn in elderly rats were lower than those in the adult rats (*P*=0.000). The two indexes were found significantly lower in the elderly COPD group and the COPD with depression group than those in the elderly control group (LSD-*t*=2.078, *P*=0.048; LSD-*t*=2.155, *P*=0.041; LSD-*t*=2.905, *P*=0.008; LSD-*t*=2.350, *P*=0.027). No significant difference was observed between elderly control and elderly depression groups (LSD-*t*=1.095, *P*=0.284; LSD-*t*=0.489, *P*=0.629) (**Fig. 2A, 2B**).

Open-field test scores in rats

There were no significant differences of the open-field test scores at baseline among groups of elderly rats

before smoking or/and CUMS. Compared to elderly control group, rats in the elderly COPD with depression group showed a significant smaller score for both horizontal movement (Kruskal Wallis test, *F*=20.59, *P*=0.005) and vertical movements (LSD-*t*=2.769, *P*=0.010) (**Fig. 2C, 2D**).

Sucrose preference in rats

The sucrose preference index in the elderly COPD groups was slight different from that in the elderly control (LSD-*t*=0.881, *P*=0.387), but there was no significance between these two groups. After exposure to CUMS for 21 days, sucrose-intake of the elderly rats in depression group and in COPD with depression group were significantly lower than rats in the elderly control group (LSD-*t*=2.091, *P*=0.047; LSD-*t*=2.138, *P*=0.043) (**Fig. 2E**).

Morris water maze test

All elderly rats showed similar time and distance to reach the platform before smoking or/and CUMS. After model establishment, the latency and traveled distance of each group to reach the platform were shown in (**Fig. 3**). Compared to the elderly control group, the time of latency and travel distance to find the platform were obviously longer in elderly COPD with depression group (LSD-*t*=-10.166, *P*=0.000; LSD-*t*=-6.448, *P*=0.000).

SOD and MDA expression in rats

Compared to the adult control group, SOD activity in both hippocampus and serum decreased significantly and the MDA expression in both hippocampus and serum increased remarkably in all elderly groups (*P*<0.05, or *P*<0.01). Meanwhile, compared to the elderly control, only the group of COPD combined with depression showed significantly reduced SOD activity in serum and hippocampus (LSD-*t*=2.629, *P*=0.014; LSD-*t*=2.125, *P*=0.044), and significantly increased MDA expression

Figure 1. Typical histopathological findings of lungs from each group of rats (stained H&E, $\times 100$).

A. Adult control group; B. elderly control group; C. elderly COPD group; D. elderly depression group; E. elderly COPD with depression group. Damaged bronchial lumen, inflammatory cell infiltration and epithelial cell proliferation were demonstrated in the elderly COPD group and the elderly COPD with depression group. COPD, chronic obstructive pulmonary disease.

Figure 2. Results of pulmonary function test, open-field test and sucrose preference test in controls and testing groups. A. FEV0.2/FVC; B. Cldyn; C. horizontal movement, expressed as median and quantile; D. vertical movement; E. sucrose preference index. # $P<0.05$, ## $P<0.01$ compared to the adult control group; * $P<0.05$, ** $P<0.01$ compared to the elderly control group. FEV0.2/FVC: ratio of forced expiratory volume at 0.2 s to the forced vital capacity; Cldyn, dynamic lung compliance; COPD, chronic obstruction pulmonary disease.

Figure 3. Results of the Morris water maze test. A. time latency; B. traveling distance. Data are showed as mean $\pm$ SD ($n=6$). # $P<0.01$ compared to the adult control group. * $P<0.01$ compared to the elderly control group.

in serum and hippocampus (LSD- $t=-2.140$, $P=0.042$; LSD- $t=-2.070$, $P=0.049$) (Table 1).

DISCUSSION

The aging population grow rapidly, and aging has become a serious issue around the world. About 46% patients of critical care and 60% patients of surgery in hospitals are the elderly.¹⁰ Researches demonstrate that over 50% of elderly people with chronic obstructive pulmonary disease (COPD) suffer from clinically significant depressive symptoms, and depression is a risk factor for mortality in stable COPD.^{11,12} Upon synchronization of the standard therapy, the level of depression in patients with exacerbation of severe COPD effectively decreases.¹³ Researchers called for further investigation into the association between COPD and depressive symptoms.^{14,15}

In the current study, model of COPD combined with depression in elderly rat was established by smoking and CUMS with solitary support. The pulmonary histology, respiratory function, open-field test and sucrose consumption of the model rats were investigated. The damaged bronchial lumen, epithelial cells and inflammatory exudate were observed in lungs of elderly rats in COPD with depression group. In addition, the index of pulmonary function, FEV0.2/FVC and Cldyn in

Table 1. SOD activities and MDA expressions in serum and hippocampus of rats in different groups [§] (n=6)

Group	Serum		Hippocampus	
	SOD (U/mL)	MDA(nmol/mL)	SOD (U/mg prot)	MDA (nmol/mg prot)
Adult control	9.50±0.48	1.24±0.36	8.16±0.47	1.17±0.20
Elderly control	7.00±0.44 ^{##}	2.41±0.35 [#]	5.20±0.45 ^{##}	2.61±0.28 [#]
Elderly COPD	5.86±0.49 ^{##}	2.85±0.33 ^{##}	4.97±0.33 ^{##}	2.82±0.44 [#]
Elderly depression	6.11±0.55 ^{##}	2.70±0.18 ^{##}	5.14±0.66 ^{##}	2.94±0.49 ^{##}
Elderly COPD with depression	5.15±0.52 ^{***}	2.94±0.49 ^{***}	3.68±0.57 ^{***}	3.85±0.60 ^{***}

§: Plus and minus values are means±SD. [#]P<0.05, ^{##}P<0.01 compared to the adult control; *P<0.05 compared to the elderly control. SOD, superoxide dismutase; MDA, malondialdehyde.

the elderly COPD with depression group were significantly lower than those in elderly control group. Open-field test showed these rats had significant decreased values of horizontal and vertical movements, and an obvious reduced sucrose-intake compared to those in elderly control group. These results were consistent with pathological changes of COPD and manifestations of depression diseases, which suggested that the comorbidity model of elderly COPD with depression was successfully established and reliable.

The oldest-old are the fastest growing age group, with the highest risk of cognitive impairment (CI).¹⁶ The concurrence of COPD and depression, especially in patients over 65 years old, poses an important hazard to cognitive function in their rest years of life. We used MWM test to assess the deficits in learning and memory in rats. Elderly rats in the group of COPD with depression showed significant longer latency and traveling distance to reach the platform than rats in the elderly control group, which mean that the combined effects of COPD and depression could induce spatial memory deficits in aging rats. Previous research had showed that CI is an important but under-recognized extra-pulmonary feature of COPD, and comorbidities such as depression was likely to synergistically contribute to the development of CI in COPD.¹⁷ Other study also found that depression comorbid with COPD increased CI as well as the severity of disease.¹⁸ Our study confirmed that COPD in comorbidity of depression could induce spatial memory deficit in old SD rats.

Recent researches have suggest that hippocampus is an important part of the brain, plays a key role in cognitive and memory function, and is also involved in the mental control of mood and mood-related thoughts.^{19,20} SOD is an important antioxidant enzyme that participate in the removal of ROS from cellular environment, while MDA, an end-product

of ROS-induced peroxidation, has been widely used as an oxidative stress biomarker.²¹ A recent study demonstrated that there were immune and non-immune inflammatory changes with oxidative stress imbalance in COPD immunopathology.²² Another study showed that oxidative stress was implicated in the pathophysiology of depression in elderly subjects.²³ In our study, we tested the SOD activity and MDA content in serum and hippocampus in model rats, and found significant decreased SOD activity and remarkable increased MDA content in both hippocampus and serum of elderly rats with COPD and concurrent depression. These results indicated that the accelerated aging effects in comorbidity of COPD and depression may be mediated by oxidative stress. This study is the first study that investigate the effects of comorbidity of COPD and depression on the cognitive impairment in rats and suggest the possible mechanism that is related to oxidative stress imbalance.

In conclusion, the present study successfully generated model of COPD combined with depression in rats using approaches of smoking and CUMS with solitary support. Significant deficits in spatial memory were observed in elderly rats with comorbidity of COPD and depression. The underlying mechanisms may relate to the overload and free radical metabolic imbalance. These results may help in a novel treatment strategy for the comorbidity of COPD and depression in elderly patients.

Conflict of interests statement

The authors have no conflict of interests disclosed.

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