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ORIGINAL ARTICLE

Increased Serum Romo1 Was Correlated with Lung Function, Inflammation, and Oxidative Stress in Chronic Obstructive Pulmonary Disease

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Abstract— Chronic obstructive pulmonary disease (COPD) is associated with abnormal inflammation and high oxidative stress. Studies suggest that reactive oxygen species modulator 1 (Romo1) involve in diseases associated with oxidative stress and inflammation. However, the relationship between COPD and Romo1 is still not clear. In this study, we compared serum Romo1 in 49 COPD patients and 34 health controls, and their correlation with lung function, systematic inflammation, and oxidative stress. In addition, serum levels of Romo1, C-reactive protein (CRP), and oxidative stress (measured by reactive oxygen species, ROS) were analyzed using commercial kits. Serum Romo1 was significantly higher in COPD patients than that of control (132.24 ± 10.34 vs. 93.26 ± 7.75 pg/ml, $P < 0.05$). Serum CRP and ROS were also significantly higher in COPD patients. Serum Romo1 was correlated inversely with FEV1% predicted in COPD patients ($r = -0.347$, $p = 0.016$), while it was correlated positively with CRP and ROS levels, respectively. These results suggest that serum Romo1 increase in COPD patients and that these levels are associated with lung function, inflammation, and oxidative stress in COPD.

KEY WORDS: chronic obstructive pulmonary disease; inflammation; oxidative stress; reactive oxygen species modulator 1.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), a worldwide health care burden, is proposed to be the third leading cause of mortality by 2030 [1, 2]. COPD with the features of progressive and partially reversible airflow obstruction is considered to be associated with abnormal airway inflammation and oxidant/antioxidant imbalance [3]. Recently, studies suggest that the inflammation may also "spread" into the circulatory system and then cause systematic inflammatory injuries and organ damage [4, 5]. On this basis, it is not appropriate to merely consider COPD as a pulmonary disease. Increased systemic inflammation may usually lead to complex chronic comorbidity (*e.g.*, coronary artery disease) in COPD patients, which then results in poor clinical outcomes [6]. Unfortunately, our understanding on the roles of systemic inflammation in COPD and its comorbidity is still limited.

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Oxidative stress is related to an imbalance between the increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and reduced antioxidant capacity [7, 8]. To our best knowledge, disturbance of the oxidant–antioxidant and proteinase–antiproteinase balance has been reported to be responsible for the pathogenesis in COPD [9]. In the presence of endogenously metabolic reactions from mitochondrial electron transport during respiration or activation of phagocytes or exogenously from air pollutants or cigarette smoke, lungs are continuously subject to oxidants [10, 11]. Air pollution–induced lung inflammation is characterized by the activation of inflammatory cells such as neutrophils, alveolar macrophages, monocytes, and epithelial and endothelial cells, which then increase inflammation and tissue damage after releasing ROS and RNS [12, 13]. ROS is required for normal cellular homeostasis and physiology in several subcellular events, such as enzyme activation, signal transduction, and gene expression [14]. In cases of disturbance of prooxidant/antioxidant equilibrium, oxidative stress is induced which then leads to damage of intracellular molecules [15].

ROS modulator 1 (Romo1), a novel protein firstly cloned from head and neck cancer tissue in 2006, is located in the mitochondrial membrane and is a key modulator of intracellular ROS [16]. To date, the elevation of circulating Romo1 has been well acknowledged in several diseases associated with high oxidative stress and inflammation, such as cancer and idiopathic pulmonary fibrosis [17, 18]. Oxidant–antioxidant imbalance in the airway played a critical role in the pathogenesis of lung injury in patients with COPD. However, the COPD pathogenesis is not well defined, chronic inflammation induced by stresses (*e.g.*, smoking) plays an important role in the onset and progression of COPD. Consistent with the links of smoking and oxidative stress, we speculated that smoking may lead to upregulation of Romo1 and release of ROS through inducing lung injury by oxidative stress, which then resulted in deterioration of lung injury. On this basis, we hypothesize that the elevation of Romo1 may play an important role in the pathogenesis of COPD. In this study, we carried out a preliminary clinical study to determine the relationship exists between COPD and Romo1.

PATIENTS AND METHODS

Patients

Forty-nine stable COPD outpatients with mild-to-very severe airflow limitation (GOLD stage I to IV) were enrolled from the COPD clinic of Nanjing First Hospital. Patients had stable disease at least for 2 months prior to their inclusion; a period free of a COPD exacerbation for 4 weeks prior to the enrollment was considered adequate to include patients in stable condition. Forty-three age-matched healthy volunteers recruited from the Physical Examination Center served as normal control. All subjects underwent a standard lung function test, and COPD was diagnosed prospectively for this study on the basis of Global Initiative for chronic obstructive lung disease criteria [19]. Written consent was obtained from each subject. The study was performed in line with the principles of the Declaration of Helsinki. The study protocols were approved by the Institutional Review Board for Human Studies of Nanjing First Hospital (Approval No. 201727003).

The inclusion criteria were as follows: those with aged ≥ 40 years; those with a ratio of forced expiratory volume in the first second to forced vital capacity (FEV1/FVC) of less than 70% after bronchodilation; those with an increase in FEV1 of less than 12% after inhalation of 훽2-agonist (200-mg salbutamol); those with clinically stable conditions for at least 3 months prior to the study. Patients with asthma, bronchiectasis, interstitial pulmonary fibrosis, hypertension, coronary artery disease, autoimmune diseases, a history of stroke, diabetes mellitus, chronic renal disease, or malignancy were excluded from the study.

Measurement of Romo1, CRP, and ROS

Subjects were required to be in a fasting state before blood collection, and then venous blood samples (5 ml) were collected from each subject followed by separation of serum and storage at − 80 °C until analysis. Serum Romo1-was measured using a human Romo1 immunoassay kit (EIAab Science, Wuhan, China) according to the manufacturer's instructions. Level of C-reactive protein (CRP) was analyzed using ELISA (Xitang Bio-Technology, Shanghai, China). Oxidative stress was assessed by measuring ROS level using a colorimetric kit (Xitang Bio-Technology, Shanghai, China). All the measurements were carried out strictly according to the manufacturer's instructions. Technicians were blinded to the clinical details of the subjects.

Statistical Analysis

Data were presented as mean ± standard deviation. Unpaired Student's *t*-test was used to determine the differences between groups in cases that the data were normally distributed. Bivariate Pearson's correlation test was used to investigate the correlations. Data were analyzed using SPSS 18.0 for Windows (IBM, Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Clinical Characteristics

In total, 49 COPD patients and 34 healthy controls were included in this study. The demographic and clinical characteristics, and smoking pack-years, as well as serum Romo1, CRP, and ROS, were shown in Table 1. No statistical differences were noticed in the age, sex, and smoking pack-years between the patients and controls. The FEV 1% among COPD patients was 85.79 ± 19.87%, which indicated that most had mild airflow limitation.

Serum Romo1, CRP, and ROS

Compared with the control, the mean serum Romo1 showed a significant increase in COPD patients (132.24 ± 10.34 pg/ml vs. 93.26 ± 7.75 pg/ml, 뭪 < 0.001; Fig. 1). Similarly, the serum CRP (6.28 ± 3.72 vs. 4.89 ± 1.15 mg/L, 뭪 = 0.02) and serum ROS (285.37 ± 85.35 vs. 237.56 ± 65.82 nmol/mL, 뭪 = 0.01) in COPD patients also showed a significant increase compared with that of the control group.

Correlations Between Romo1 and FEV1%, CRP, and ROS

Serum Romo1 in COPD patients was negatively correlated with lung function, based on FEV1% ($r = -0.460$, $P = 0.001$; Fig. 2a). In addition, serum Romo1 was also negatively correlated with the ratio of FEV1/FVC ($r = -0.523$, $P = 0.003$). Whereas, Romo1 was positively correlated with CRP ($r = 0.367$, $P = 0.009$; Fig. 2b) and ROS ($r = 0.487$, $P < 0.001$; Fig. 2c) in COPD patients.

DISCUSSION

For the first time, we investigated the correlations between serum Romo1in COPD patients and indicators of lung function, inflammation, and oxidative stress. Our study showed that significant correlations were noticed between Romo1 levels and FEV1%, CRP, and ROS. In future, further studies are needed to justify the mechanistic basis of these correlations to testify whether Romo1 levels contribute to the pathogenesis of COPD.

There is a strong biologic rationale as to why Romo1 may have a modulatory role on lung function in COPD. To date, several genetic markers of oxidative stress have been reported to involve in the development of COPD. In pathologic conditions, the imbalance between excess formation and/or impaired removal of ROS may lead to ROS accumulation [20]. Mitochondria were considered the major source of ROS, and the mitochondrial ROS modulator 1 (Romo1) was reported to be involved in ROS production. In a previous study, Lee and colleagues suggested that Romo1-derived ROS might play an important role in the necrosis of apoptotic cell [21]. Meanwhile, Chung et al. demonstrated that Romo1 was responsible for the excessive accumulation of ROS in tumor cells [16]. Moreover, the authors reported that mitochondrial ROS generated by Romo1 expression was required for cell proliferation, which suggested that Romo1 may play a crucial role in redox signaling during normal cell proliferation [22]. Whereas, in a previous study, ROS trigger lung injury and contributed to lung diseases such as COPD, acute lung injury, and cancer [23–25], which suggested that ROS may be associated with the pathogenesis of COPD. Thus, Romo1 may contribute to the COPD lung by triggering ROS

Table 1. Characteristics of Patients with COPD and Healthy Subjects

Characteristic	COPD ($n = 49$)	Control ($n = 34$)	P value
Age (year)	63 ± 9	59 ± 10	0.178
Sex(m/f)	33/15	22/10	0.027*
BMI (kg/m2)	22.58 ± 3.26	23.05 ± 2.64	0.368
Smoking (pack years)	14.83 ± 15.48	12.07 ± 18.72	0.468
FEV1(L)	2.07 ± 0.64	2.78 ± 0.58	< 0.001
FVC(L)	3.42 ± 1.28	3.26 ± 0.65	0.348
FEV1/FVC%	58.74 ± 7.35	84.57 ± 6.06	< 0.001
FEV1% predicted	85.79 ± 19.87	116.49 ± 16.32	< 0.001
Romo1(pg/ml)	132.24 ± 10.34	93.26 ± 7.75	< 0.001
CRP (mg/L)	6.28 ± 3.72	4.89 ± 1.15	0.02
ROS (nmol/ml)	285.37 ± 85.35	237.56 ± 65.82	0.01

COPD, chronic obstructive pulmonary disease; *CRP*, C-reactive protein; *FEV1*, forced expiratory volume in 1 s; *FVC*, forced vital capacity; *Romo1*, reactive oxygen species modulator 1; *ROS*, reactive oxygen species

* The chi-squared test was used to test the significance of the difference in gender proportions.

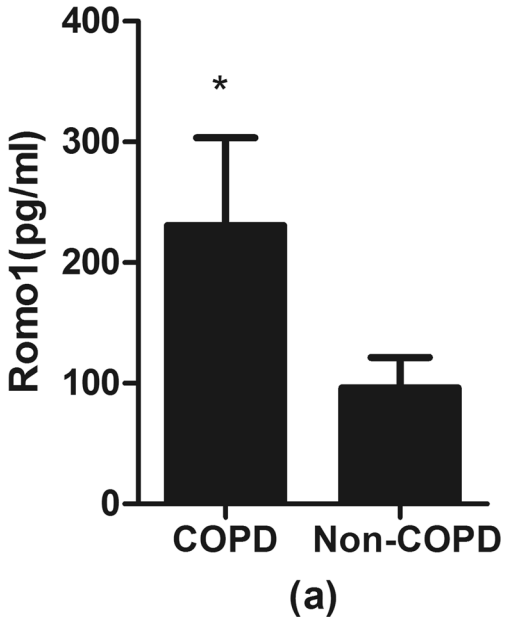

Fig. 1. Comparison of serum Romo1 level in COPD patients compared with the non-COPD individuals. The data were presented as mean ± standard error. *$P < 0.05$ versus non-COPD control.

accumulation. Taken together, it is reasonable to speculate that Romo1 may participate in the pathogenesis of COPD.

In this study, serum CRP and ROS showed a significant increase in COPD patients compared with that of controls. Circulating CRP was elevated in COPD patients and may thus be regarded as a valid biomarker of systemic inflammation. Additionally, serum CRP was associated with the decline of FEV1. These results suggested that CRP and ROS may contribute to the chronic inflammation and oxidative stress of COPD. What's more, a negative correlation was observed between Romo1 and lung function in COPD patients, which demonstrated that Romo1may correlate with the severity of airway obstruction. Previous studies reported that smoking over the clinical course of COPD may lead to the production of Romo1; however, in our study, no statistical differences were noticed in the smoking between the two groups. In future, more studies are needed to clarify whether smoking may contribute to Romo1levels and COPD in general.

Our study has a few limitations. Firstly, this research was a single-center study, and the sample size of the subgroups was relatively small. Secondly, relatively few research indicators were included, only two indicators were utilized to analyze systemic inflammation and oxidative stress. Thirdly, the differences in expression of Romo1 in sputum, bronchoalveolar lavage fluid, airway epithelial cells, and lung tissue were no studied in depth. In future, these indices would be determined, together with *in vitro* experiments. Meanwhile, cigarette smoke extract was used to stimulate the epithelial cells of the bronchus, and then Romo1 expression and function were determined to assess the roles of smoking in COPD-related inflammation and oxidative stress. Furthermore, most of our patients presented with relatively mild COPD. Our subsequent study will verify our findings in patients with more severity, as well as determination of Romo1 expression in the presence of smoking, before and after treatment.

In summary, serum Romo1 is significantly increased in COPD patients, which is associated with lung function, inflammation, and oxidative stress in these patients. Accordingly, it has the potential to be used as a biomarker of disease progression and cardiac comorbidity in COPD patients. In future, studies are needed to examine additional markers of systemic inflammation and oxidative stress in order to understand the roles of Romo1 in the pathogenesis of COPD.

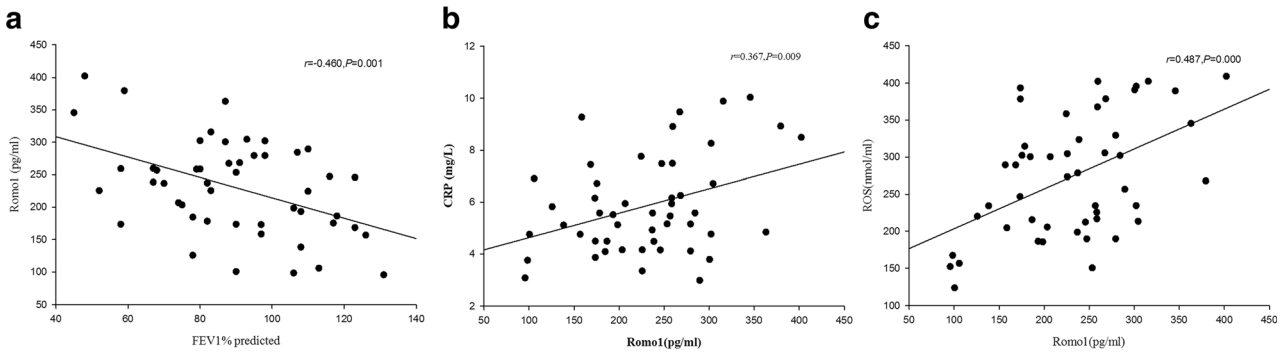

Fig. 2. Correlation between Romo1 and FEV1% (a), CRP (b), and ROS (c).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

Ethical Approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent. Informed consent was obtained from all individual participants included in the study.

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