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Mechanisms of oxidative stress effects of the NADPH oxidase-ROS-NF-κB transduction pathway and VPO1 on patients with chronic obstructive pulmonary disease combined with pulmonary hypertension

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Abstract. – OBJECTIVE: This study explored the oxidative stress effects of the NADPH oxidase-ROS-NF-κB transduction pathway and vascular peroxidase 1 (VPO1) on patients with chronic obstructive pulmonary disease (COPD) combined with pulmonary hypertension (PH).

PATIENTS AND METHODS: 30 patients with pure COPD and 30 patients with stable COPD combined with PH, who were admitted to our hospital from January 2015 to December 2015, were enrolled as the control group and the observation group, respectively. NADPH oxidase activity in mononuclear cells, reactive oxygen species (ROS), NF-κB, VPO1 levels in serum, and further oxidative stress-related indices were compared and analyzed.

RESULTS: Systolic pulmonary pressure (SPAP) (63.65±9.47 mmHg) of the observation group was higher than SPAP (53.13±7.52 mmHg) of the control group (p<0.05); malondialdehyde (MDA) (7.81±2.24 nmol/l) of the observation group was lower than MDA (9.54±3.38 nmol/l) of the control group (p<0.05); superoxide dismutase (SOD) (406.73±147.35 U/ml) of the observation group was higher than SOD (295.16±106.73 U/ml) of the control group (p<0.05). NADPH oxidase activity, ROS and NF-κB levels of the observation group were higher than those of the control group (p<0.05). The average level of serum VPO1 in the observation group (236.71±42.35) ng/L was significantly higher than that of the control group (122.56±34.62) ng/L (p<0.05). In the observation group, the level of SPAP was positively correlated with the concentration of VPO1 (r=0.615, p<0.05), the serum levels VPO1 were negatively correlated with MDA levels (r=-0.537, p<0.05), MDA and ROS levels were negatively correlated (r=-0.482, p<0.05), and the levels of SOD were positively correlated with NF-κB (r=0.427, p<0.05).

CONCLUSIONS: The NADPH oxidase-ROS-NF-κB transduction pathway, VPO1, oxidative stress and their complicated interactions are implicated in the occurrence and development of COPD combined with PH.

Key Words:
Chronic obstructive pulmonary disease, Pulmonary hypertension, NF-κB, Vascular peroxidase 1, Oxidative stress.

Introduction

The morbidity and mortality rates of chronic obstructive pulmonary disease (COPD) are increasing gradually. Evidence[1,2] has shown COPD to be the third leading cause of mortality in the world. Pulmonary hypertension (PH) is an important factor for patients with COPD whose condition further develops into pulmonary heart disease[3]. Once it combines with PH, COPD greatly increases adverse health risks to patients, whose quality of life and prognosis are significantly reduced[4]. As the pathogenesis of COPD combined with PH has not yet been elucidated, it may be insightful to assess the potential role that oxidative stress plays in its mechanisms of causation[5-8]. This work investigates the oxidative effects of the NADPH oxidase-ROS-NF-κB transduction pathway and vascular peroxidase 1 (VPO1) on patients with COPD combined with PH. It is hoped that evidence from this study may provide a valuable reference for the formulation of clinical drug treatment for these patients.

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Patients and Methods

Patients

30 patients with stable COPD combined with PH, who were admitted in our hospital from Jan. 2015 to Dec. 2015, were selected as the observation group. Among this group, there were 22 males and 8 females, with an average age of 66.5 (±3.3) years old, and 23 smokers, with an average smoking rate of 46.7 (±2.4) packs per year. 30 patients with pure COPD were also selected as the control group. Among this group, there were 21 males and 9 females, with an average age of 65.8 (±3.1) years old, in which there were 21 smokers, with an average smoking rate of 44.2 (±2.6) packs per year. The criteria created by the Respiratory Medicine Group of the Chinese Medical Association were adopted as the COPD diagnostic criteria. The PH diagnostic criterion was defined as pulmonary arterial systolic pressure (PASP) > 40 mmHg. For both groups, cases with complications of the liver, heart, hypertension, or the kidney or other comorbidities were excluded. The demographic data of two groups were compared, and none of the differences had statistical significance. This study was approved by the Ethics Committee of The First Affiliated Hospital of Northwest University for Nationalities. Signed written informed consents were obtained from all participants.

Methods

3 ml of blood was drawn from the patients on an empty stomach for the two groups. Blood samples were handled with anticoagulation protocol and centrifugal separation at 2000 r/min, and were stored under -70°C. Reactive oxygen species (ROS) levels were measured by colorimetric methods. The VPO1 levels of patients' plasma were determined by Western blot. The NF-κB levels of plasma were determined by ELISA methods, and the kit was provided by Shanghai Yanyu Biotechnology (Co., Ltd, Shanghai, China). Superoxide dismutase (SOD) was measured by xanthine oxidase methods, and the kit was provided by Shanghai Solarbio Bioscience & Technology (Co., Ltd, Shanghai, China). Malondialdehyde (MDA) content was tested by thiobarbituric acid methods, and the kit was provided by Shanghai Xinyu Bio-engineering (Co., Ltd, Shanghai, China).

Cardiac color ultrasound (Philips, Eindhoven, The Netherlands) was applied to estimate the pulmonary artery pressure of the patients in two groups and pulmonary systolic pressure (SPAP) was calculated using the simplified Bernoulli equation: SPAP (mmHg) = PG (PGTI) + 10 mmHg. Lymphocyte-separation medium was used to separate the mononuclear cells in blood by density gradient, and the fluorescence method was used to measure the activity of NADPH oxidase. Lymphocyte separation medium was offered by Shanghai Bosheng Bioscience & Technology (Co., Ltd, Shanghai, China), and the enzymatic activity was presented as nmol/min/mg protein.

The correlations of NADPH oxidase activity, ROS, NF-κB, VPO1 levels, and oxidative stress-related indices of the two groups were then calculated, compared and analyzed.

Statistical Analysis

Software SPSS (Version X; IBM, Armonk, NY, USA) 20.0 was used to tabulate and analyze the data. Measurements were indicated as mean values with ± standard deviation. Analyses were carried out using *t*-tests or X^2 analysis. Correlational analysis was conducted by applying the Pearson method. For all tests, $p<0.05$ indicated that the differences had statistical significance.

Results

Comparison of Oxidative Stress Indicators, SPAP, MDA and SOD Between the two Groups

SPAP values of 63.65 (±9.47) mmHg of the observation group were higher than SPAP of 53.13 (±7.52) mmHg of the control group ($p<0.05$); MDA values of 7.81 (±2.24) nmol/L of the observation group were lower than MDA of 9.54 (±3.38) nmol/L of the control group ($p<0.05$); and SOD values of 406.73 (±147.35 U/ml) of the observation group were higher than SOD of 295.16 (±106.73U/ml) of the control group ($p<0.05$) (Table I).

Comparison of NADPH Oxidase, ROS and NF-Kb Indices Between the two Groups

NADPH oxidase activity (7.13±0.46), ROS (0.754±0.156) and NF-κB (0.662±0.027) levels of the observation group were each respectively higher than those of the control group (3.74±0.15, 0.538±0.103, and 0.312±0.015) and each difference was statistically significant ($p<0.05$) (Table II).

Table I. Oxidative stress indicators, SPAP, MDA and SOD.

Groups	N	SPAP (mmHg)	MDA (nmol/l)	SOD (U/ml)
Observation Group	50	63.65±9.47*	7.81±2.24*	406.73±147.35*
Control Group	50	53.13±7.52	9.54±3.38	295.16±106.73

*Differences between the observation group and the control group were statistically significant (p<0.05).

Table II. Comparison of NADPH Oxidase, ROS and NF-κB indices between the two groups.

Groups	N	NADPH oxidase activity (nmol/min/mg)	ROS (U/l)	NF-κB (ng/l)
Observation Group	50	7.13±0.46*	0.754±0.156*	0.662±0.027*
Control Group	50	3.74±0.15	0.538±0.103	0.312±0.015

* Differences between the observation group and the control group were statistically significant (p<0.05).

Comparison of Plasma VPO1 Levels Between the two Groups

The average level of plasma VPO1 in the observation group was 236.71 (±42.35) ng/l, which was significantly higher than that of the control group 122.56 (±34.62) ng/l (p<0.05) (Figure 1).

Correlation Analysis of SPAP and VPO1

In the observation group, the levels of SPAP were positively correlated with the concentrations of VPO1 (r=0.615, p<0.05) (Figure 2).

Correlation Analysis of MDA and VPO1

In the observation group, the plasma VPO1 concentrations were negatively correlated with MDA concentrations (r=-0.537, p<0.05) (Figure 3).

Correlation Analysis of MDA and ROS

In the observation group, MDA and ROS levels were negatively correlated (r=-0.482, p< 0.05) (Figure 4).

Correlation Analysis of SOD and NF-κB

In the observation group, the levels of SOD were positively correlated with NF-κB (r=0.427, p<0.05) (Figure 5).

Discussion

COPD poses a serious threat to human health, while COPD combined with PH has an even

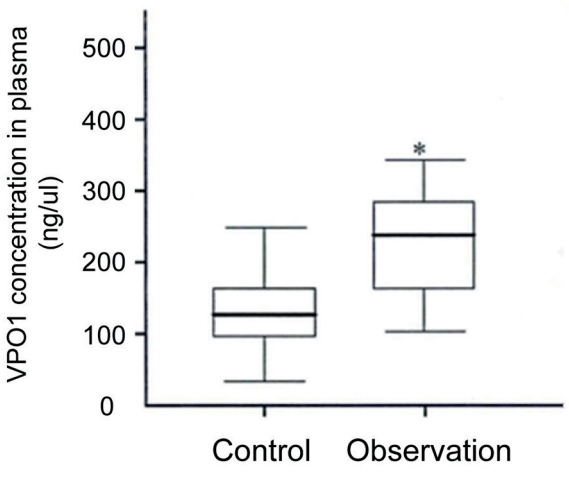

Figure 1. Comparison of plasma VPO1 levels between the two groups. *The difference between the two groups was statistically significant (p<0.05).

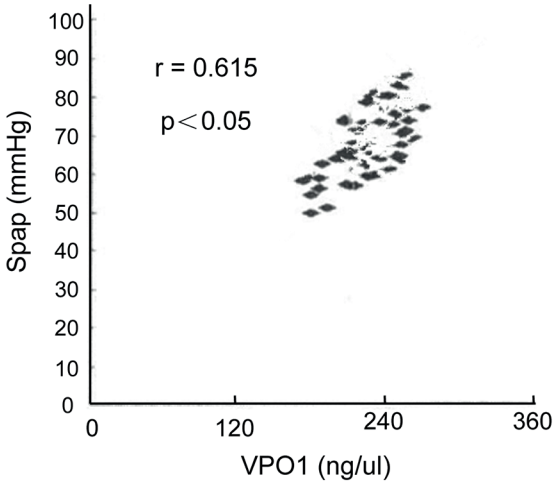

Figure 2. Correlation analysis of SPAP and VPO1 Measures of SPAP are plotted against levels of VPO1 among the observation group, showing a positive correlation.

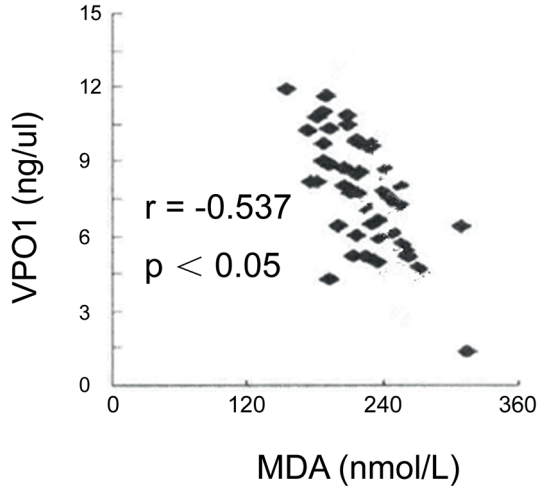

Figure 3. Correlation analysis of MDA and VPO1. Levels of MDA are plotted against levels of VPO1 among the observation group, showing a negative correlation.

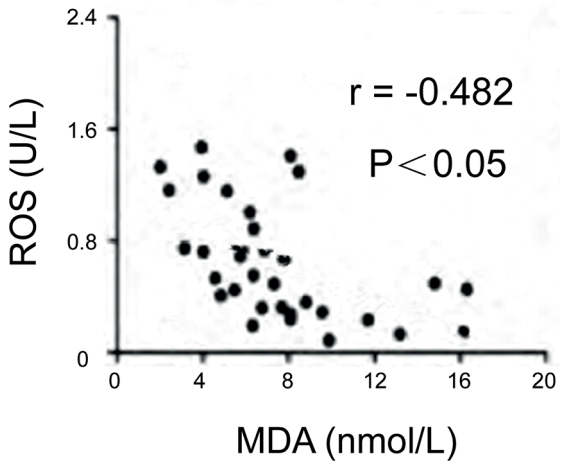

Figure 4. Correlation analysis of MDA and ROS. Levels of MDA are plotted against levels of ROS among the observation group, showing a negative correlation.

higher mortality rate than COPD alone[9]. Therefore, patients with COPD should be monitored closely to enable timely discovery of PH and the reduction of its occurrence, to decrease complications from congestive heart failure and to reduce the mortality rate of COPD. The major manifestations of PH are a gradual incrassation of pulmonary endangium, a gradual stenosis of the pulmonary vasoganglion and an increasing resistance of the pulmonary blood vessels[10-13] Each of these processes is implicated in inflammatory reactions and oxidative stress.

COPD combined with PH is a complicated process that is influenced by many factors. Some research holds that oxidative stress can promote the occurrence and development of PH[14]. Oxidative stress involves an accumulation of ROS and active nitrogen species (RNS), as occurs when the body is exposed to harmful and adverse stimulations and disease states, leading to an imbalance of the antioxidant system and induction of tissue damage[15-17]. However, to maintain redox equilibrium in the human body, superoxide dismutase, and other endogenous antioxidant proteins act to stabilize these reactions by eliminating ROS and RNS and thereby protecting cells and tissues from the adverse effects of oxidative stress.

Recently it was discovered that NF-κB is a nuclear transcription factor which can activate and regulate the expressions of multiple inflammatory factors[18]. The transcription of antioxidant genes mediated by the NADPH oxidase-ROS-NF-κB transduction pathway is an important mechanism for cells to respond to oxidative stress. While ROS can bring harms to cells through the oxidation of intracellular structures, it can also activate oxidative stress response pathways by increasing antioxidant activity, to alleviate tissue damage[19].

This study showed that SPAP of the observation group was higher than SPAP of the control group (p<0.05); MDA of the observation group was lower than MDA of the control group (p<0.05); and SOD of the observation group was higher than SOD of the control group (p<0.05), which suggested that, compared to patients with COPD alone, the oxidative stress among patients with COPD combined with PH was more pronounced.

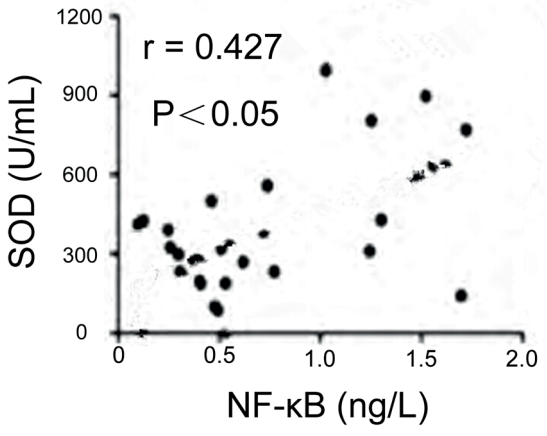

Figure 5. Correlation Analysis of SOD and NF-κB. Levels of SOD are plotted against levels of NF-κB among the observation group, showing a positive correlation.

This research also demonstrated that NADPH oxidase activity, ROS and NF-κB levels of the observation group, were higher than those of the control group ($p<0.05$). In the observation group, MDA and ROS levels were negatively correlated (r=-0.482, $p<0.05$) and levels of SOD were positively correlated with NF-κB (r=0.427, $p<0.05$), which indicated that the NADPH oxidase-ROS-NF-κB transduction pathway participated in the oxidative stress response to the disease state and was implicated in the formative process of COPD's combination with PH.

VPO1 is an important peroxidase that is expressed predominantly in the heart and vascular walls. It has a certain acceleration function in vascular smooth muscle cell proliferation and plays a crucial role in vascular diseases through the induction of oxidative stress[20,21]. In this study, the average level of plasma VPO1 in the observation group was 236.71 (±42.35) ng/l, which was significantly higher than that of the control group (122.56±34.62 ng/l) ($p<0.05$). Pearson correlation analysis showed that the level of SPAP in the observation group was positively correlated with the concentration of VPO1 (r=0.615, $p<0.05$), which further supported VPO1's close association with oxidative stress. Its increase could stimulate oxidative stress and thereby contribute to the occurrence and pathogenesis of COPD with PH.

Conclusions

We have found evidence that both the NADPH oxidase-ROS-NF-κB transduction pathway and VPO1 may be actively involved in the oxidative stress-induced pathology of COPD combined with PH. These oxidative stimuli may increase the antioxidant stress response by reducing pulmonary arterial pressure and eliminating SOD and other indices, providing valuable references for the prevention and therapeutic treatment of COPD combined with PH.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest.

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