The effects of antioxidants on exercise-induced lipid peroxidation in patients with COPD

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Objective: The oxidant–antioxidant balance plays an important role in the pathogenesis of COPD. The aim of the present study was to evaluate the effects of exercise, as an oxidative stress factor on the oxidant–antioxidant balance and to investigate whether short-term antioxidant treatment affects lipid peroxidation products.

Methodology: Twenty-one stable COPD patients and 10 control subjects were included in the study. Symptom-limited exercise tests were performed by all subjects. Blood was collected before and 1 h after exercise in control subjects and before, 1 and 3 h after exercise in COPD patients, for analysis of malondialdehyde (MDA), reduced glutathione (GSH) and vitamin E (VE) levels. VE and vitamin C treatments were added to the regular bronchodilator therapy in 10 COPD patients for 1 month. After the treatment period, an exercise test was performed and blood was collected again for MDA, GSH and VE levels.

Results: Baseline GSH and VE levels were significantly lower in the COPD group when compared with the control subjects. There was no statistically significant difference in MDA levels between the two groups. In the COPD group, MDA levels 3 h after exercise were significantly higher than at baseline. In contrast there were no significant differences in MDA, VE and GSH levels in the control group after exercise. VE and MDA levels increased significantly after exercise in COPD patients but there was no difference in GSH levels. Baseline exercise time was significantly lower in the COPD group than in the controls. In 10 COPD patients who were given antioxidant therapy, their exercise time increased significantly and there was no increase in MDA and VE levels after the repeated exercise test.

Conclusions: Antioxidant levels were significantly lower in COPD patients than in control subjects. In these patients, exercise results in more significant oxidative stress and lipid peroxidation than in control subjects and antioxidant therapy may decrease lipid peroxidation following exercise and improve exercise capacity.

Key words: COPD, exercise, glutathione, lipid peroxidation, malondialdehyde, vitamin E.

INTRODUCTION

Oxidative stress can be defined as an increased exposure to oxidants and/or decreased antioxidant capacity. It is recognized as an important factor in the pathogenesis of many diseases.1 There is strong evidence that COPD is one of these diseases and increased oxidative stress is an important pathogenic factor for COPD.2–5 Superoxide anion (O2•−), hydroxyl radical (OH•), hydrogen peroxide (H2O2) and hypochlorous acid are some of the important and potent oxidant agents. Antioxidants can be separated into two groups: enzymatic and non-enzymatic. The major enzymatic oxidants are superoxide dismutase (SOD), catalase and glutathione (GSH). Vitamin E (VE), vitamin C (VC), α-carotene and uric acid are some of the non-enzymatic factors that may function as antioxidants.1,4 Smoking,
environmental pollution, infections and exercise can affect the balance between these systems and may cause oxidative stress. Oxidative stress produces irreversible damage to DNA and various cell constituents, a deficiency in antioxidants, inactivation of antiproteases and lipid peroxidation. Consequently, all of these pathological changes contribute to the pathogenesis of COPD.

Because lipid peroxidation is an outcome of oxidative stress, numerous markers have been used to monitor lipid peroxidation. Malondialdehyde (MDA) is one of the more important lipid peroxidation products that can be shown to increase following oxidative stress.

This model of oxidant stress and tissue damage in COPD leads us to hypothesize that there are benefits from antioxidant therapy in patients with COPD.

The aim of the present study was to compare the effects of exercise-induced oxidative stress on lipid peroxidation in COPD patients and healthy controls and to investigate the effect of antioxidant therapy on these parameters.

METHODS

Study population

Twenty-one stable COPD patients and 10 age- and gender-matched healthy controls were included in the study. Patients were excluded if they had been admitted to hospital with an exacerbation in last 3 months, had reversible airflow obstruction, were insufficiently mobile to perform an exercise test and/or had cardiovascular disease other than that secondary to COPD.

The study was approved by the Ethics Committee of the Medical Faculty of the Kocaeli University, Kocaeli, Turkey and all participants gave informed consent.

Study design

Symptoms, duration of disease, smoking history and medication use were recorded and a physical examination was performed. Pulmonary function (PFT) and arterial blood gases (ABG) were analyzed. Patients were asked to perform a symptom-limited exercise test on the second day of the study. The exercise tests were continued until either the patient reported fatigue or dyspnoea or ST/T wave abnormalities were seen on their electrocardiogram. Before exercise and 1 and 3 h after its cessation, blood was collected from a peripheral vein for later analysis of MDA, GSH and VE.

As with the patient group, a complete medical history was taken and a physical examination performed in the controls. Symptom-limited exercise tests were performed and blood was collected before exercise and 1 h after exercise cessation for MDA, GSH and VE measurements. The subjects whose exercise test had significant ST/T wave abnormalities were excluded from the study.

Ten of 21 patients from the COPD group were given VE (200 IU/day) and VC (500 mg/day) tablets for 1 month. After this treatment period, the same exercise test protocol was performed and blood was collected for GSH, MDA and VE analyses.

MDA, GSH and VE measurements

Fasting blood samples were collected in the morning, from an antecubital vein without stasis from trained subjects before exercise and 1 and 3 h after cessation of exercise. Venous blood samples were collected in vacutainer plain tubes containing disodium EDTA (1.5 mg/mL) for GSH, and MDA measurements. A 2-ml aliquot was removed to determine Hb, hematocrit (Hct) and erythrocyte (RBC) count. Samples were immediately centrifuged (900 g for 10 min at 4°C), and serum samples were used for VE measurements. Whole blood and plasma samples were used for GSH and MDA measurements, respectively.

Lipid peroxidation levels were monitored by determining the end product of lipid peroxidation, MDA by the thiobarbituric acid method. Plasma MDA values were calculated using the extinction coefficient of MDA-thiobarbituric acid complex (532 nm = 1.56 × 10³ mol/cm) and expressed as nmol/mL. GSH levels were assayed using the method of Beutler et al. In brief the non-protein sulphhydryl groups of RBCs are in the form of reduced GSH. 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) is a disulphide chromogen. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration. The GSH concentration was expressed in mg/dL of RBC. Serum VE concentrations were measured using the method described by Varley et al. and expressed as mg/dL.

Exercise test

All patients and the control group underwent a graded treadmill exercise test using the Bruce protocol. Symptom-limited exercise tests were performed with a Quinton 710 machine. Most of the exercise tests were terminated due to marked fatigue. The total exercise time and maximal workload were determined for all subjects. Patients who had a positive cardiological test response were excluded from the study.

Statistical analysis

All data were expressed as mean ± SEM. Statistical analysis of comparison between COPD and control groups was performed using Student's t-test. A P-value < 0.05 was considered to be significant.

RESULTS

Patients in the COPD group were males with a mean age of 62.7 ± 9 years. Ten healthy men were selected as
a control group and the mean age in this group was 49.5 ± 6 years. The mean smoking period in the COPD group was 49 ± 24 pack-years and disease duration was 6.5 ± 6.4 years. Demographic features of the study subjects are shown in Table 1.

All of the COPD patients were receiving a combination of bronchodilator therapy (which included inhaled anticholinergic drugs), oral slow-release theophylline and inhaled long-acting β-2 agonist.

Baseline GSH and VE levels were significantly lower in the COPD group than in the controls, while there was no significant difference between MDA levels in the two groups (Table 2).

In the COPD group, both the MDA and VE levels increased significantly with exercise. The MDA levels at 3 h after exercise cessation were significantly higher than the baseline values. Similarly, VE levels 1 h after exercise were found to be significantly higher than the baseline levels. However, there was no statistically significant difference in GSH levels after exercise (Fig. 1).

In the control group, exercise did not cause any significant differences in MDA, GSH and VE levels.

The comparable levels of MDA, GSH and VE after 1 month of VE and VC treatments are shown in Table 3. Although baseline and after-exercise levels of these variables did not show any significant difference, the previously described increase in MDA and VE levels following exercise did not occur after the second exercise test.

Table 2 Comparison of oxidant–antioxidant levels before treatment

<table>
<thead>
<tr>
<th></th>
<th>COPD (n = 21)</th>
<th>Control (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH 0 (mg/dL)</td>
<td>8.37 ± 7</td>
<td>22.43 ± 16.6</td>
<td>0.02*</td>
</tr>
<tr>
<td>GSH 1 (mg/dL)</td>
<td>6.29 ± 4.8</td>
<td>28.6 ± 6.2</td>
<td>0.002*</td>
</tr>
<tr>
<td>MDA 0 (nmol/mL)</td>
<td>0.63 ± 0.27</td>
<td>0.63 ± 0.27</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MDA 1 (nmol/mL)</td>
<td>0.53 ± 0.3</td>
<td>0.58 ± 0.28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VE 0 (mg/dL)</td>
<td>5.09 ± 2.8</td>
<td>7.6 ± 2.9</td>
<td>0.02*</td>
</tr>
<tr>
<td>VE 1 (mg/dL)</td>
<td>6.82 ± 3.6</td>
<td>10.8 ± 7</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

0, Before exercise; 1, 1 h after exercise cessation. *P < 0.05, statistically significant.

Table 3 Before and after treatment levels of malondialdehyde (MDA), glutathione (GSH) and vitamin E (VE) in COPD patients (n = 10)

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH 0 (mg/dL)</td>
<td>8.5 ± 7.7</td>
<td>6.2 ± 5.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GSH 1 (mg/dL)</td>
<td>5.0 ± 3.1</td>
<td>7.5 ± 3.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GSH 3 (mg/dL)</td>
<td>9.4 ± 8.2</td>
<td>8.9 ± 3.5</td>
<td>&gt;0.05</td>
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<tr>
<td>MDA 0 (nmol/mL)</td>
<td>0.75 ± 0.2</td>
<td>0.98 ± 0.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MDA 1 (nmol/mL)</td>
<td>0.57 ± 0.3</td>
<td>0.84 ± 0.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MDA 3 (nmol/mL)</td>
<td>0.94 ± 0.5</td>
<td>0.98 ± 0.6</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VE 0 (mg/dL)</td>
<td>4.8 ± 3.3</td>
<td>8.6 ± 3.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VE 1 (mg/dL)</td>
<td>6.8 ± 3.6</td>
<td>6.9 ± 4.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VE 3 (mg/dL)</td>
<td>5.3 ± 2.5</td>
<td>7.2 ± 2.1</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

0, Before exercise; 1, 1 h after exercise cessation; 3, 3 h after exercise cessation.

Figure 1 (a) Malondialdehyde (MDA), (b) glutathione (GSH) and (c) vitamin E (VE) in COPD during exercise test before treatment (n = 10). P < 0.05: Statistically significant.
Lipid peroxidation in COPD

DISCUSSION

COPD is characterized by progressive and irreversible airway obstruction. Smoking, alpha-1 antitrypsin deficiency and occupational exposures are known to be aetiological factors. Although the mechanisms of airflow obstruction are not clearly understood, it is considered that oxidant–antioxidant imbalance plays an important role in the pathogenesis of COPD.1

The aim of the present study was to compare the effects of exercise on oxidant–antioxidant balance in COPD patients and healthy controls. We also intended to determine whether it was possible to decrease exercise-induced oxidative stress with short-term antioxidant therapy.

MDA, a lipid peroxidation product, was used as a marker of oxidative stress. Although there was no significant difference in MDA levels between COPD and control groups, exercise caused a significant increase only in the COPD patients. Previously, it has been reported that the effect of exercise on lipid peroxidation was minimal and that exercise did not cause a significant increase in MDA levels in healthy controls, which is similar to the present findings.16 However, our data have confirmed that exercise-induced lipid peroxidation is significant in COPD compared with control subjects.

GSH is a well-known extracellular antioxidant that protects tissues from the effects of oxidative stress.17 GSH levels decrease during exacerbation of COPD9 and in our study GSH levels were found to be low in stable COPD patients. These data suggest that the oxidant–antioxidant balance was altered in favour of oxidants, even in stable COPD.

Heunks et al. investigated the effects of exercise on MDA levels and the oxidized glutathione (GSSG) to GSH ratio in COPD, and found that exercise caused significant increases in both MDA levels and GSSG/GSH ratio.18 In the present study, we observed a significant increase in MDA levels following exercise, while the increase in GSH levels was not statistically significant. The difference between Heunks’ study and the present study suggests that the GSSG/GSH ratio may change without there being a significant difference in the GSH levels. We could not measure this ratio in the present study and therefore future studies will be needed to clarify this difference.

We used VE as well as GSH to measure the antioxidant capacity. Before-exercise VE levels were significantly higher in the control group compared to the COPD group. It is postulated that this difference in VE is associated with oxidative stress. Significantly, exercise caused an increase in VE levels only in patients with COPD. This finding suggests that VE may also play an important role in preventing exercise-induced lipid peroxidation. The increase in VE levels was thought to be associated with an increase in MDA levels. As we failed to find a significant difference in GSH levels following exercise, it seems important to assess the GSSG/GSH ratio and not just GSH alone.

Because the oxidant–antioxidant imbalance is an important pathogenetic feature of COPD, the possible beneficial effects of antioxidant therapy have been discussed. Therefore, we investigated the effects of VE and VC on exercise-induced lipid peroxidation in the second stage of the study. MDA, GSH and VE levels did not show any difference after treatment. However, we observed that exercise did not cause any significant increase in MDA levels, which was the opposite of the pretreatment findings. We also demonstrated that antioxidant therapy improved exercise capacity in COPD. These data caused us to hypothesize that it might be possible to decrease the harmful effects of oxidative stress with antioxidant therapy. Similar findings have been reported elsewhere.5,7,19

Antioxidant therapy has not been recommended for patients with COPD. Future studies that include more patients and a much longer treatment period are needed to further define the effects of antioxidant therapy on oxidative stress in COPD.

REFERENCES

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