Tanaffos (2008) 7(3), 13-17

Enhanced Cytochrome C Oxidase Activity in WBC of COPD Patients

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ABSTRACT

Background: *Chronic obstructive pulmonary disease (COPD) is characterized by decreased expiratory flow rates, increased pulmonary resistance and hyperinflation. Cytochrome C Oxidase (COX) as a key oxidative enzyme modulates oxygen uptake and catalyzes the oxidation of reduced cytochrome C by molecular oxygen. In vitro studies indicate that the activity of COX can be directly regulated by the presence of molecular oxygen. Thus, a better understanding of the role of COX in patients with COPD can provide an important link between the availability of oxygen to tissues and the regulation of oxygen uptake and energy production in these patients.*

Materials and Methods: *We studied 42 COPD patients (36 males, 6 females) with clinically stable conditions and 50 (42 males, 8 females) healthy sedentary volunteers of similar age. Whole blood was collected by venipuncture in sodium citrate tubes and WBCs were separated by Ficoll according to standard protocol and lysed with microtube pestle homogenizer. The homogenates were centrifuged and the supernatants were used as a cell extract for COX activity determination. Aliquots of this were assayed for total protein content and COX activity. Analysis of COX activity was performed using COX assay kit. Absolute specific COX activity was normalized for total protein. Relative activities were determined by dividing absolute specific COX activity on absolute specific citrate synthase activity.*

Results: *Mitochondrial COX activity and specific activity (absolute and relative) significantly increased in WBCs of patients with COPD in comparison with control samples ($p< 0.05$).*

Conclusion: *These results indicated that the activity of COX was increased in WBCs of patients with COPD but whether this is a primary or secondary change relevant to hypoxic condition in these patients is not clear and needs further investigation.*

(Tanaffos 2008; 7(3): 13-17)

Key words*: COPD, Cytochrome C oxidase (COX), Mitochondria, Specific activity*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), a common disease characterized by a poorly reversible limitation in airflow, is predicted to become the third most frequent cause of death in the world by 2020 (1, 2).The risk of death in patients with COPD is often assessed by the use of a single physiological variable, the forced expiratory volume in one second (FEV1) (1, 3, 4). One of the major problems in these patients

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Received: 17 September
Accepted: 23 July 2008

is insufficient absorption and consumption of oxygen (5). Most of the consumed oxygen is processed by the cytochrome C oxidase (COX). COX is the terminal oxidase of cellular respiration catalyzing the transfer of electrons from reduced cytochrome c to molecular oxygen in mitochondria (6).

The primary aim of this study was to assess COX activity in patients with COPD compared to normal subjects in a noninvasive manner.

MATERIALS AND METHODS

Population

We studied 42 COPD patients (36 males, 6 females, mean age 60 ± 2 yrs) with clinically stable conditions (all were smokers and had been suffering from an acute exacerbation during the past 4 months or more), and 50 healthy (42 males, 8 females, mean age 56 ± 4 yrs) sedentary volunteers of similar age (Table 1). We studied only patients without known neuromuscular disorders, heart failure, diabetes mellitus, alcoholism and whose condition was exacerbated. All of them signed an informed consent, after awareness of the nature, characteristics, and risks of the study. This investigation was approved by the local ethics committee of the National Research Institute of Tuberculosis and Lung Disease (NRITLD) of Masih Daneshvari hospital, Shahid Bsheshti University, M.C., Tehran.

Lung function assessment

Pulmonary function tests (PFT) were performed in all patients .We used some parameters for the assessment of lung function and grading of COPD. Forced expiratory volume produced in first second (FEV1) and forced vital capacity (FVC) were measured in all participants (7). Spirometric reference values were from a Mediterranean population (8). The patients were categorized as having mild COPD (FEV1/FVC<70%, FEV 1≥80% predicted), Moderate COPD (FEV1/FVC<70%, 50%≤FEV1<80% predicted), Severe COPD (FEV1/FVC<70%, 30%≤FEV1<50% predicted) and normal (FEV1>75%, FVC% predicted>75%, FEV1/FVC>70%).

Table1. Mean (±SD) of different variables measured in the study.

Variables	Control	COPD
Age, yrs.	56±4	60±2
FEV1, % reference	90±5	34±3*
FEV1/FVC, %	82±2	43±4*
Total protein content, mg/ml cell extract	6±2	6±2
absolute Specific activity of COX [A] nmol.min^{-1}.mg^{-1} of cell extract protein	22±2	79±4*
absolute Specific activity of citrate synthase [B] nmol.min^{-1}.mg^{-1} of cell extract protein	80±5	80±5
%Relative activities {[A]/ [B]}.100	27±0.9	98±8*

* Significance of differences with respect to control subjects.

Preparing samples

White blood cells (WBC) were chosen for mitochondrial assay as it is a standard procedure for the investigation of respiratory chain disorders in humans (9) and is a minimally invasive test. Whole blood was collected by venipuncture in sodium citrate tubes. Blood (7–9 ml) was layered on 9 ml Ficoll–Paque® Plus (Pharmacia Biotech) in a 50 ml centrifuge tube and centrifuged at 400×*g* for 30 min. The WBC layer was transferred to a clean 50 ml centrifuge tube and washed twice using a balanced salt solution and then centrifuged at 150×*g* for 10 min. Precipitates were resuspended in 200 µl lysis buffer (10mM Tris-HCl, pH 7.0, containing 250mM sucrose and 1mM n-dodecyl-β-D-maltoside) and lysed with microtube pestle homogenizer (Medi-Cal, cat.#6211). N-dodecyl-β-D-maltoside is a nonionic detergent with an alkyl chain as a lipophilic moiety and by using this detergent, the active cytochrome C oxidase was solubilized from mitochondria (10). The homogenates were centrifuged for 10 min at 8000 g, and the supernatant was stored at −20 °C for future use as a cell extract which possesses mitochondrial proteins (11).

COX Activity Determination

All cell extracts were defreezed and held in an ice bath. Aliquots of these homogenates were assayed for total protein content (12) and COX activity (E.C.1.9.3.1). Analysis of COX activity was performed using COX Assay kit (Sigma cat# CYTOCOX1), according to its protocol in a spectrophotometer with continuous optical absorbency registry (Shimadzu, Nagoya, Japan). Specific COX activity was normalized for total protein. All reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Relative Activities Determination

For proportioning mitochondrial content with absolute specific COX activity in all samples, relative activities were established dividing absolute specific COX activity by absolute specific citrate synthase activity (13), which was assessed spectrophotometrically (14).

Analysis of citrate synthase activity was performed using citrate synthase assay kit (Sigma cat# CS0720) according to its protocol.

Statistical Analysis

Mean and standard deviation (SD) and percentages were used for quantitative and qualitative variables, respectively. Comparisons between results were carried out using a paired t-test after assessing the normality of the distribution (Kolmogorov-Smirnov's test) and the equality of variances (Levene's test). Values of $p<0.05$ were considered as statistically significant.

RESULTS

All patients had severe airflow limitation (Table 1). Total protein content in cell extract was similar (≈6 mg/ml of cell extract) in patients and normal subjects. The absolute specific activity of COX ($nmol.min^{-1}.mg^{-1}$ of cell extract protein) was higher in patients than in normal subjects (Table1).The absolute specific activity of citrate synthase ($nmol.min^{-1}.mg^{-1}$ of cell extract protein) was about 80 in both groups (nonsignificant difference)as demonstrated with respect to specific activity of COX (Figure 1).

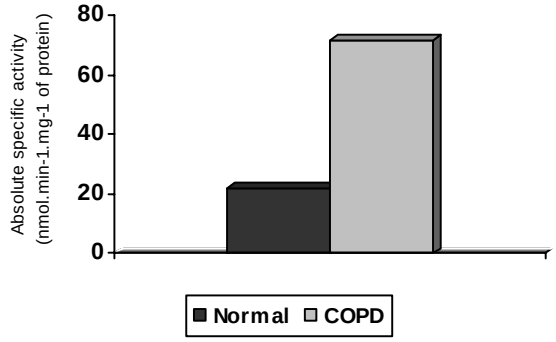

Figure 1. Mean ±SD absolute specific activity of COX between the two groups.

As mentioned, the relative activity was calculated and showed significant differences ($p< 0.05$) between patients and normal subjects. Actually, relative activity increased about 3.5 times in patients in comparison with normal subjects (Figure 2) and had a similar pattern of increase in absolute specific activity of COX associated with COPD.

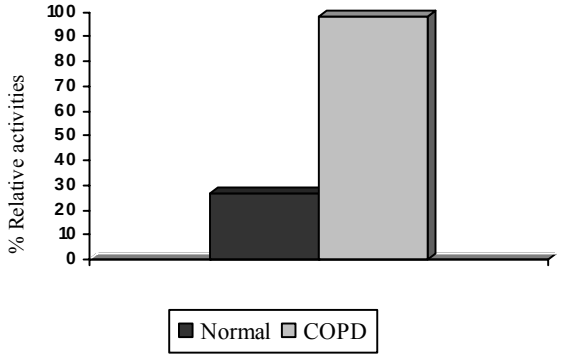

Figure 2. Mean ±SD % relative activities.

DISCUSSION

Our study results indicated that the absolute and relative activities of COX increased in WBCs of patients with COPD as compared to normal subjects.

The specific relevance of this increase in COX activity in WBCs of these patients is not clear, but increasing the potential velocity or enzymatic content or gene expression levels of this enzyme are probably involved in a primary response or compensatory reactions of the cells to hypoxic conditions.

Other pervious studies have also shown increased COX activity in skeletal muscles of patients with peripheral arterial insufficiency under conditions of tissue hypoxia (15, 16) in healthy individuals at moderate altitudes (14,17,18) and in Patients with COPD (19).

We found one study evaluating COX activity in peripheral blood mononuclear cells (PBMC). The results of this study showed that COX activity decreased following acute smoking (20).

It should be noted that the most important risk factor for development of COPD is chronic smoking (21). Thus, it is expected that in response to long-term smoking and hypoxic conditions, rising levels of COX activity is a remedial mechanism. Furthermore, our findings offer an explanation to why patients with COPD often show higher oxygen consumption than control subjects with the same workload.

To ascertain that the observed increased COX activity in COPD patients is due to hypoxic conditions, it would be best to investigate the degree of increased activity of this enzyme in these patients without prior smoking.

Evaluation of COX activity in WBC of these patients, in a noninvasive manner, could also open a new avenue in research work in these patients.

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