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SHORT COMMUNICATION

ANTIOXIDANT RESPONSE TO OXIDATIVE STRESS INDUCED BY SMOKING

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Abstract : Oxygen free radicals have been hypothesized to play a pivotal role in the deleterious effects of smoking on health. The present study was undertaken to examine the oxidant and antioxidant system among smokers and nonsmokers. Fourteen smokers and 11 nonsmokers were enrolled for this study. The protein carbonyl levels in smokers were found to be significantly higher than in nonsmokers. The levels of plasma ascorbic acid, free sulfhydryl group, and erythrocyte reduced glutathione were lower in smokers compared to nonsmokers. In smokers the erythrocyte activities of both glutathione peroxidase and catalase were decreased when compared to that in nonsmokers. The data from the study reemphasizes the presence of oxidative stress in smokers. The concomitant decrease in the activities of both catalase and glutathione peroxidase found in the erythrocytes of smokers raises rational grounds for expressing concern over the increased susceptibility towards oxidative stress in these subjects.

Key words : smoking glutathione peroxidase catalase oxidative stress carbonylation

INTRODUCTION

Cigarette smoking is a serious health problem worldwide (1). Smoking has been strongly implicated as a risk factor for chronic obstructive pulmonary disease, cancer, and atherosclerosis (2–4). Because cigarette smoke is known to contain a large number of oxidants, it has been hypothesized that many of the adverse effects of smoking may result from oxidative damage to critical biological substances (5). Such damage could result both from oxidants present in cigarette smoke and from the activation of phagocytic cells that generate reactive oxygen species (5).

Biological effects of these highly reactive compounds are controlled *in vivo* by a wide spectrum of antioxidant mechanisms (6).

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Among these, the enzymes glutathione reductase and catalase act as important endogenous antioxidants. Antioxidant enzymes play a crucial role in determining individual risk of developing certain diseases, such as cancer and atherosclerosis (6).

The pathophysiology of oxygen radicals has been examined extensively in cigarette smokers (7–9), with the focus placed on quantifying antioxidant depletions or measuring oxidatively altered proteins, lipids, and DNA; studying the effects of antioxidant supplementation on these indexes; and studying perturbations in these indexes after smoking cessation (8, 9).

In spite of the circumstantial data from *in vitro* studies and measurements of antioxidant levels in smokers suggesting that cigarette smoke may cause oxidative injury, the literature is controversial, as illustrated by the discordance over the erythrocyte levels of glutathione peroxidase (GSH-Px; EC 1.11.1.9) and catalase (CAT; EC 1.11.1.6) in smokers (10–13).

As in other countries, in India smokers require particular attention in terms of public health interventions. Since, wide interindividual variations may exist regarding antioxidant capacity, thus affecting individual susceptibility against deleterious oxidative reactions, the purpose of our study was to examine the activities of erythrocyte glutathione peroxidase and catalase and plasma concentrations of indexes of antioxidant defenses and oxidative stress in a sample population of healthy men consisting of current smokers and nonsmokers.

MATERIALS AND METHODS

Fourteen males who had smoked more than 10 cigarettes for more than 10 years and 11 individuals who had never smoked participated in the study. All subjects were healthy individuals who were not receiving current medication or nutritional supplements and who had no history of cardiovascular, endocrine, or gastrointestinal disorders.

Overnight fasting blood samples were taken by venipuncture in tubes containing EDTA. One milliliter of the collected sample was used for the estimation of reduced glutathione (GSH) and hemoglobin (Hb). The rest of the sample was centrifuged (2500 × g 10 minutes at 4°C). The plasma obtained was used for the estimation of total ascorbic acid, MDA, protein carbonyls and free sulfhydryl group. The samples were processed immediately after collection to minimize the possibility of oxidation of ascorbic acid, free sulfhydryl and reduced glutathione. Samples were stored at –80°C for the estimation of protein carbonyl.

Whole blood reduced glutathione was estimated with Ellman's reagent and the results were expressed as mg per gram of hemoglobin (4). The glutathione peroxidase activity was measured by the method of Rotruck et al (15). The RBC catalase activity was determined by the method of Beers and Sizer (16). The method is based on monitoring the rate of decomposition of H_2O_2. The decrease in optical density at 240 nm was measured. Plasma total ascorbic acid concentration was determined using 2,4-dinitrophenyl hydrazine (7) Plasma free

sulfhydryl group was estimated according to the method of Hu et al (18). Carbonylation of plasma protein were estimated by the method according to Reznick and Packer (19).

Student's t-test was used to assess the significance of difference. Differences were considered significant at $P<0.05$.

RESULTS

Table I shows the erythrocyte antioxidant enzyme activities and glutathione levels along with oxidative stress indices of plasma in smokers and healthy controls. Catalase activity was significantly decreased in smokers when compared with controls. Erythrocyte from smokers showed a decreased glutathione peroxidase activity than that of control subjects. When the level of reduced glutathione was compared between smokers and nonsmokers, a significant decrease in the levels of reduced glutathione was observed in smokers. The concentration of total ascorbic acid in plasma was lower in smokers than in nonsmokers.

TABLE I: Levels of oxidants and antioxidant parameters in healthy smokers and nonsmokers.[a]

	Nonsmokers	*Smokers*
Age (year)	38.18±3.37	38.86±4.35
Reduced glutathione (mg/g Hb)	8.13±1.34	6.29±1.28*
Catalase (U/g Hb)	104.40±15.19	53.90±8.82*
Glutathione peroxidase (U/g Hb)	45.10±10.34	31.36±3.63*
Protein carbonylation (nmole/mg protein)	0.54±0.25	1.26±0.13*
Ascorbic acid (mg/I)	14.34±2.86	11.59±1.80*
Free sulfhydryl group (nmole/I)	559.63±25.86	460.97±34.14*

[a]Data are expressed as mean±S.D.
*$P<0.01$ compared to controls subjects.

Protein carbonyl content in the smokers was increased significantly when compared to that in control. The protein thiol level in smokers was decreased significantly when compared to that in the control.

DISCUSSION

The sustained inhalation of reactive free radicals in the gas and tar phases of tobacco (5) imposes an oxidant stress and perturbs the antioxidant defense systems in blood and tissue of smokers (7). Among the elaborate antioxidant defense system of erythrocyte, catalase and glutathione peroxidase play an important role in neutralizing the deleterious effects of H_2O_2 and other peroxides.

Even though there is fairly irrefutable evidence for oxidative stress in cigarette smokers, both the simultaneous decrease in glutathione peroxidase and catalase observed in the present study is not a common finding. While some investigators observed a decrease in glutathione peroxidase levels, with normal catalase activity (10), others have recorded an increase in glutathione peroxidase activity with a decrease in catalase activity in the erythrocytes of smokers when compared with non-smoking healthy individuals (11). It has also been reported that the activity of catalase in erythrocyte of smokers is increased with unaltered glutathione peroxidase activity (12). Durak et al have reported that the enzymatic activity of both glutathione peroxidase and catalase in erythrocyte of smokers are similar to healthy control (13). The simultaneous decrease in activities of both these antioxidant enzymes as observed in the present study may be detrimental to the body as this can lead to the accumulation of H_2O_2 in their erythrocytes, making them

prone to damage by iron-mediated formation of oxyradicals. Cigarette are said to be good source of oxidants including iron in good quantity (5).

Although we cannot explain this contradictory finding, but as these enzymes arc found to be influenced by genetic factors it can be hypothesized that Indians are more susceptible for such changes. These paradoxical findings among smokers recorded by various investigators may also be due to the fact that some smokers do not inhale the smoke from their own cigarettes. As smokers are often exposed for longer periods to cigarette smoke from other smokers, the recorded number of cigarettes smoked by an individual may be a poor estimate for the actual exposure to the smoke toxins.

The results from other oxidative stress parameters reemphasizes the findings from a large body of evidence that has been marshaled in the past decade, to support the presence of oxidative stress in smokers. This argument is buttressed by the fact that cigarette smoke contains a plethora of potential reactive oxygen and nitrogen species (5).

As reported by other investigators (13), this study has also demonstrated reduced levels of GSH in erythrocyte of smokers. The reduced form of the tripeptide thiol, glutathione (GSH) is one of the major endogenous defense mechanism against oxidative stress. Thus, GSH depletion can further hamper cellular defenses against oxidative stress.

Our estimate of the effect of smoking on circulating concentrations of vitamin C generally agrees with the conclusions of other studies of lowered plasma vitamin C concentrations in smokers (20, 21). This association has been found to persist despite correction for factors that independently affects plasma ascorbic acid concentrations, such as alcohol and vitamin intakes (21). This observation suggests that smoking directly lowers plasma vitamin C concentrations by mechanisms that do not depend on dietary vitamin C intake, such as impaired vitamin C absorption or decreased turnover (20, 21). For protein – SH groups in plasma (Table I), our data are consistent with previous report that cigarette smoking causes oxidation of protein – SH groups in plasma.

Reactive oxygen species can damage all types of biological molecules. Oxidative damages to proteins, lipids or DNA may all be seriously deleterious and may be concomitant (6). However, proteins are possibly the most immediate vehicle for inflicting oxidative damage. Protein carbonyls are considered as markers of oxidative stress induced protein damage. Our result of increased protein carbonyl in plasma of smoker's was in accord with that reported previously (8).

In conclusion, the concomitant decrease in the activities of both catalase and glutathione peroxidase in the erythrocytes of smokers raises rational grounds for expressing concern over the increased susceptibility towards oxidative stress in these subjects. Smokers develop increased plasma protein oxidation as shown by decrease in thiol and increased carbonyl values in the present study. Future epidemiological studies to identify genes whose expression can be altered by smoking are warranted.

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