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Effects of Cigarette Smoking on Blood Antioxidant Status in Short-Term and Long-Term Smokers

Abstract: To determine the effects of cigarette smoking on blood antioxidant status and lipid peroxidation, 16 healthy male current smokers (CS) and 16 healthy male non-smokers (NS) serving as controls were studied. CS were divided into two groups: short-term smokers (STS) 35.4 ± 5.8 years of age (mean±SD), and long-term smokers (LTS) 60.9 ± 4.9 years of age. The average smoking period of STS and LTS were 8.1 ± 1.1 years (mean ±SD) and 20.5 ± 4.5 years (mean ± SD)respectively. STS and LTS had their own controls, who were NS (young NS and old NS respectively).

When STS and LTS values are compared with their control values, the following are determined:
1-SOD and catalase activities increased significantly ($p < 0.05$) in LTS, but were unchanged in STS.
2-STS and its control contained the same quantity of total glutathione and reduced glutathione (GSH). However, the oxidized glutathione (GSSG) level in STS was significantly elevated ($p < 0.01$).

3- In LTS, reduced glutathione diminished ($p < 0.01$), while GSSG increased significantly ($p < 0.01$), but total GSH was unchanged.
4-Smoking did not affect a-tocopherol level in STS, but an apparent decrease was observed in LTS ($p < 0.05$).
5-Thiobarbituric acid reactive substance (TBARs) level as an index of lipid peroxidation increased significantly ($p < 0.05$) in LTS, in spite of being unchanged in STS.
6-In comparing short-term and long-term smokers to each other, significant decreases were observed in total glutathione, reduced glutathione and α-tocopherol levels ($p < 0.05$) in LTS. There were no significant differences between STS and LTS in the other measured parameters.

It was concluded that the changes in the enzymatic and nonenzymatic antioxidant defense systems of elderly smokers may be due to oxidative stress caused by cigarette smoking.

Key Words: Oxidative stress, antioxidants, TBARs

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Introduction

The oxidant stress from cigarette smoking is substantial. A puff of smoke contains 10^{15} oxidant radicals equally distributed between the gases and the particles (1). Cigarette smoke is known to stimulate the alveolar macrophages (AMs) to release excessive levels of free radicals, which are believed to play a role in the development of chronic bronchitis, emphysema and inflammatory diseases (2). Moldeus et al. (3) indicated that tobacco smoke oxidants severely deplete intracellular antioxidants in lung cells by a mechanism that may be related to increased oxidant stress. Similar depletion of antioxidants in whole animal lungs and alveolar lavage cells has been demonstrated by Cotgreave et al. (4). It is likely that the free radicals inhaled with smoke and the increased levels of oxygen derivatives generated in the lung of smokers by phagocytes will enter the circulation and modulate the antioxidant enzyme activities of blood (5). However, the effects of cigarette smoke exposure on antioxidant enzyme activities are controversial. Jendryczko et al. (6) observed significant decreases in the antioxidant capacity of erythrocytes in passive smokers. Toth et al. (7) hypothesized that the antioxidant activities and protective abilities of erythrocytes in cigarette smokers might be increased compared with erythrocytes in non-smokers. In addition, Abou-Seif (8) found that erythrocyte SOD and catalase activities were elevated in smokers.

The variability of the effects of smoking on antioxidant enzyme activities may be due to multiple reasons, such as interaction between direct and passive smoke exposures, different smoking patterns, and differences in the compositions of cigarettes.

Because of the discrepancy in results, we believe that it is necessary to examine the effects of smoking period on blood oxidant and antioxidant status in humans. However, there has been no comparative study carried out on the effect of short-term and long-term smoking on this subject. Thus, the purpose of the present study was to determine whether short-term and long-term smoking caused significant changes in blood antioxidant status and lipid peroxidation in healthy humans.

Methods

Subjects and sampling procedures. Sixteen healthy male volunteers who were CS and 16 male NS used as control subjects were studied, as shown in Table 1. CS were assigned to two groups: STS, 26 to 42 years of age (mean ± SD, 35.4 ± 5.8), and LTS, 51 to 65 years of age (mean ± SD, 60.9 ± 4.9). The STS had a mean cigarette consumption of > 1 pack/day for less than 10 years (8.1 ± 1.1 mean ±SD), and the LTS had a mean cigarette consumption of > 1 pack/day for more than 15 years (20.5 ± 4.5 mean ± SD). Among the NS, there was no smoking history, and the physical characteristics of these subjects were similar to those of the CS (Table 1). All subjects had no evidence of acute infection, and none of them had received medication during the previous year. There were no abnormal findings in peripheral blood chemistry or electrocardiographic, chest radiographic, and spirographic examinations. The study was approved by the Ethics Committee of the University of Dicle, and all subjects signed an informed consent document prior to entering the study. The study was carried out during fasting time in the morning. Blood was withdrawn by syringe without stasis from an antecubital vein in each subject and immediately transferred to heparinized glass tubes. There was no evidence of hemolysis in any of the samples. All analyses were completed within a few hours of collection of the samples.

Measurements. Activity of red cell Cu-Zn superoxide dismutase (Cu-Zn SOD) was measured by the method of Winterbourn et al. (9), which is based on the inhibition of the reduction of nitroblue tetrazolium (Sigma Chemical Company, P.O. Box 14508, St. Louis, MO, U.S.A.) by O_2 produced via photoreduction of riboflavin (Sigma). Fifty percent inhibition was defined as 1 unit of SOD activity. Catalase activity was assayed in hemolysates of erythrocytes by monitoring the consumption of H_2O_2 at 240 nm as described by Aebi (10). Total glutathione (GSH+GSSG), oxidized glutathione (GSSG) and reduced glutathione (GSH) in erythrocytes were determined by the glutathione reductase-DTNB [5,5'-Dithiobis-(2-nitrobenzoic acid)] assay of Tietze (11). Serum α-tocopherol concentration was determined by high pressure liquid chromatography (HPLC) (12). Serum lipid peroxide concentration was measured as the total thiobarbituric acid reactive substances (TBARS) as described by Asakawa and Matsushita (13).

Statistical Analysis. The Mann-Whitney U test was used to analyze the differences between smokers' and non-smokers' values.

Results

There was no significant difference in the physical characteristics between the CS and NS groups (Table 1).

Antioxidant enzyme activities and glutathione. To examine the effects of smoking on blood antioxidant capacity, we measured erythrocyte SOD, catalase enzyme activities total glutathione, reduced glutathione, and GSSG levels. A significant increase was observed for GSSG in erythrocyte from STS as compared with those from Young-NS (p<0.01). No significant changes were observed in the other quantities mentioned above in STS (Table 2). The activities of SOD and catalase were enhanced significantly in LTS as compared with those

Table 1.   Physical characteristics of the study subjects

	(Young) Non-smokers	(STS) Short-term smokers	(Old) Non-smokers	(LTS) Long-term smokers
Age (year)	32.1± 6.2	35.4 ±5.8	57.8 ± 3.2	60.9 ± 4.9
Height (cm)	176.2 ±8.7	174.3 ± 9.2	175.4 ± 6.9	172.5 ±6.3
Body mass (kg)	70.4 ± 7.8	67.6 ± 8.1	68.3 ± 5.8	64.4 ± 6.5
Smoking period (year)	-	8.1 ± 1.1	-	20.5 ± 4.5

Values are expressed as mean ± SD

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from Old-NS (p<0.05). Significant differences were found in reduced glutathione and GSSG from LTS as compared with those from Old-NS (p<0.01).

Serum α-tocopherol and TBARS. There were no significant changes in α-tocopherol, an antioxidant vitamin, and TBARS, which is an index of lipid peroxidation, in STS. However, a decrease in α-tocopherol and an increase in TBARs levels were found to be statistically significant (p<0.05) in LTS. Additionally, in order to examine whether smoking period has an effect on blood antioxidant status and lipid peroxidation, we compared the groups STS and LTS. Significant decreases in total glutathione, reduced glutathione and α-tocopherol concentrations were found in LTS as compared with STS (p<0.05) (Table 2).

Discussion

Our findings related to the activities of SOD and catalase indicated that erythrocytes from LTS had higher enzyme activities than did erythrocytes from old NS, whereas changes were not statistically significant in STS. We observed that erythrocyte reduced glutathione levels decreased significantly in LTS (p<0.01). However, there were no apparent changes in STS. GSSG levels significantly increased in STS and LTS (p<0.01). In normal human erythrocytes, the levels of oxidized glutathione are low (14), and any elevation of GSSG suggests a pathological process (6). The basis for the increased concentrations of antioxidant enzymes in RBC from cigarette smokers is unknown. However, it has been speculated that

changes in RBC antioxidant enzyme activities are a response to increased numbers of alveolar macrophages and neutrophils that appear to be releasing increased amounts of O_2 metabolites, such as H_2O_2 or H_2O_2-derived products (15,16). It has been reported that exposure to oxygen radicals induces an increase in the lung antioxidant defense capabilities (17). McCusker and Hoidal (18) indicated that there is a selective increase in the activities of SOD and catalase but not in those of GPx in the AMs of cigarette smokers.

A previous study indicated that the SOD activity and GSSG level of RBC increased significantly in passive smokers (6). Interestingly, similar results were observed in LTS in the present study. Catalase activity, GSH and vitamin E concentrations of RBC were unchanged in passive smokers whereas in our study GSH and vitamin E concentrations decreased while catalase activity increased in LTS. A possible explanation for these differences in results may be that the subjects employed in their study were young and passive smokers.

It was reported that erythrocytes from healthy cigarette smokers contain more glutathione and catalase, and protect lung endothelial cells from H_2O_2 better than do erythrocytes from age- and gender-matched non-smokers (7). In the present study, catalase activity was similar to that reported in Toth et al. (7), but glutathione levels were different in cigarette smokers. We studied two smoker groups separately and observed the changes in LTS. Thus, the conflicting results with those of Toth et al. (7) with regard to glutathione may be due to differences

Table 2.   Erythrocyte antioxidants, antioxidant enzyme activities and TBARs in smokers and non-smokers

	Young Non-smokers (n=8) Median	Short term smokers (n=8) Median	Old Non-smokers (n=8) Median	Long term smokers (n=8) Median
SOD(IU.gHb-1)	1734.00	1741.50	1705.00	1751.50 *
Catalase(k.gHb-1)	1456.50	1493.50	1437.50	1494.00 *
T.GSH (mg.gHb-1)	1.10	1.10	0.95	0.76 ✛
GSH(mg.gHb-1)	0.97	0.87	0.83	0.51 ** ✛
GSSG(mg.gHb-1)	0.11	0.20 **	0.09	0.23 **
TBARs(nMol..ml-1)	1.81	1.91	1.82	2.08 *
Vit.E (mmol/L)	14.66	13.75	14.05	11.07 * ✛

*p<0.05, **p<0.01: The statistical comparison is between current smokers and their control values.
✛, p<0.05; The statistical comparison is between long-term smokers and short-term smokers values.

in the physical characteristics and smoking period of the subjects. McCusker and Hoidal (18) indicated that activities of SOD and catalase from the AMs of cigarette smokers and smoke exposed hamsters were twice those found in control subjects. Increases in erythrocyte antioxidant enzymes may also be parallel to increases in lung or other tissue antioxidant enzymes. If the latter is true, then increases in erythrocyte antioxidants may be effective, easily accessible indicators of oxidant stress and/or may reflect increases in other cells and tissues antioxidant concentrations. Therefore, it is highly plausible that our results show similarities with those from McCusker and Hoidal (18). In addition, Abou-Seif (8) observed lower plasma vitamin E levels and higher erythrocyte SOD and catalase activities in cigarette smokers in comparison with nonsmokers. These results are consistent with our data obtained from LTS. However, another study pointed out increased production of oxygen radical species, and decreases in antioxidant activity were observed in AMs from smokers versus those from non-smokers (16). Similar depletion of antioxidants in whole animal lungs and alveolar lavage cells has been demonstrated, but it is likely that this depletion results from chemical conjugation of antioxidants rather than from increased oxidant stress (4). According to the results of Durak et al. (19), smoking did not affect activities of SOD, catalase and GSH-Px in erythrocytes whereas plasma TBARS increased significantly in smokers. Additionally, Durak et al. (19) indicated that smoking caused no impairment in the enzymatic antioxidant defense system, because erythrocytes have a potent defense capacity. These findings were similar to our results obtained from STS. Moreover, we observed that the enzymatic antioxidant defense capacity of erythrocytes was augmented in LTS. In order investigate whether cigarette smoking would affect serum a-tocopherol concentration and TBARs levels as an index of lipid peroxidation, we evaluated these parameters in smokers and non-smokers. The elevated serum TBARs levels and reduced a-tocopherol concentration suggest that lipid

peroxidation is enhanced in LTS. Similar results were demonstrated by Jendryczko et al. (6) in passive smokers. In addition, Zhou et al. (20) found lower plasma vitamin E concentrations and elevated plasma and erythrocyte lipoperoxide levels in smokers with longer smoking duration. The same researchers indicated that the SOD, catalase and GSH-Px activities of erythrocytes were significantly lower in smokers than in non-smokers. Bellizzi et al. (21) determined that red blood cells from smokers contained less alpha-tocopherol than those from non-smokers, despite plasma levels of alpha-tocopherol being similar in smokers and non-smokers. Bingöl et al. (22) suggested that chronic smoking causes peroxidation reactions in both plasma and erythrocytes. In another study (23) it was indicated that cigarette smokers had significantly higher rates of in vivo (breath ethane) and in vitro (serum TBARS) lipid peroxidation.

In our opinion, there is no substantial agreement on erythrocyte antioxidant parameters in smokers. The conflicting results in the studies might arise from methodological differences. The present study was carried out with a relatively small number of subjects. It would be better to perform this study with a greater number of subjects in order to determine the differences between the analysis parameters of smoker and non-smoker groups more clearly.

The clinical importance of the present results suggests that free radicals inhaled in cigarette smoke are highly toxic, and impaired oxidant-antioxidant balance is a risk factor in degenerative diseases.

In summary, we conclude that cigarette smoking, especially long-term smoking may lead to significant changes in the enzymatic and non-enzymatic antioxidant defense systems of elderly smokers. As a result of the changed antioxidant status in long-term smokers, peroxidation reactions may be accelerated and some deleterious changes may occur in the body of the smoker.

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