EFFECTS OF CIGARETTE SMOKING ON BLOOD ANTIOXIDANT STATUS AND LIPID PEROXIDATION

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ABSTRACT

To determine the effects of cigarette smoking on the blood antioxidant status and lipid peroxidation, 16 healthy male current smokers (CS) and 16 healthy male non-smokers (NS) served as controls were studied. CS were divided into two groups as short-term smokers (STS) 35.4±5.8 yr of age (mean±SD) and long-term smokers (LTS) 60.9±4.9 yr of age. STS and LTS had own Controls who were NS (Young NS and Old NS respectively). We found that SOD and catalase activities increased significantly (p<0.05) in LTS, but unchanged in STS. STS and its control contained the same quantity of total glutathione and reduced glutathione (GSH). However, oxidized glutathione (GSSG) level in STS elevated significantly (p<0.01). Reduced glutathione diminished (p<0.01), while GSSG increased significantly (p<0.01) but total GSH unchanged in LTS as compared with those from old NS. To our findings, smoking did not affect α-tocopherol level in STS, but apparent decrease was observed in LTS (p<0.05). Thiobarbituric acid reactive substance (TBARs) level as an index of lipid peroxidation increased significantly (p<0.05) in LTS, in spite of unchanged in STS. In comparison with short-term and long-term smokers to each other, significant decrease were observed in total glutathione, reduced glutathione and α-tocopherol levels (p<0.05) in LTS.

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

Key words: Oxidative stress - antioxidants - TBARs

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 (AMs) macrophages to release excessive levels of the 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 into the circulation and will modulate the antioxidant enzyme activities of blood (5). However, the effect of cigarette smoke exposure on antioxidant enzyme activities are controversial. Jendryczko et al. (6) observed significant decreases in antioxidant capacity of erythrocytes in passive smokers. Toth et al. (7) hypothesized that the antioxidant activities and protective abilities of erythrocytes from cigarette smokers might be increased compared with erythrocytes from 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 of smokers, and differences in the composition of cigarettes.

Because of the discrepancy in results, we believed that it is necessary to examine the effects of smoking period on blood oxidant and antioxidant status in humans. However, there has been no study carried out comparatively 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 on blood antioxidant status and lipid peroxidation in healthy humans.

MATERIAL AND 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 into two groups as 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 had no smoking history, and physical characteristics of these subjects were similar to those of the CS (table-1). All subjects had no evidence of acute infection, and non-of them had received medication during the previous year. They showed no abnormal findings, peripheral blood chemistry, electrocardiographic, chest radiographic, and spirometric examinations. Investigation was approved by the Ethical Committee of the University of Dicle and all subjects signed an informed consent document prior to entering the study. The study was carried out at fasting time in the morning. Blood was withdrawn by syringe without stasis from an antecubital vein of each subjects 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 superoxide dismutase (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 O2 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 H2O2 at 240 nm as described by Aebi (10). Total Glutathione (GSH+GSSG), oxidized glutathione (GSSG), and reduced glutathione (GSH) were determined by the glutathione reductase-DTNB [5,5'-Dithiobis-(2-nitrobenzoic acid)] assay of Tietze (11). Serum a-tocopherol concentration was determined by high pressure liquid chromatography (HPLC) (12). Lipid peroxide concentration was measured as the total thiobarbituric acid reactive substances (TBARS) as described by Asakawa and Matsushita (13).

Statistical Analysis. Mann Whitney U test was used to analyse 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).

Table 1. Physical characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>(Young)</th>
<th>(STS)</th>
<th>(Old)</th>
<th>(LTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-smokers</td>
<td>Short term smokers</td>
<td>Non-smokers</td>
<td>Long term smokers</td>
</tr>
<tr>
<td>Age (year)</td>
<td>32.1 ± 6.2</td>
<td>35.4 ± 5.6</td>
<td>57.8 ± 3.2</td>
<td>60.9 ± 4.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.2 ± 8.7</td>
<td>174.3 ± 9.2</td>
<td>175.4 ± 6.9</td>
<td>172.5 ± 6.3</td>
</tr>
<tr>
<td>Body mass(kg)</td>
<td>70.4 ± 7.8</td>
<td>67.6 ± 8.1</td>
<td>68.3 ± 5.8</td>
<td>64.4 ± 6.5</td>
</tr>
<tr>
<td>Smoking period (year)</td>
<td>-</td>
<td>8.1 ± 1.1</td>
<td>-</td>
<td>20.5 ± 4.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

Antioxidant enzyme activities and glutathione. To examine the effects of smoking on blood antioxidant capacity, we measured erythrocyte SOD, and catalase enzyme activities and total glutathione, reduced glutathione, and GSSG levels. 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 other quantities mentioned above in STS(table-2). The activity of SOD and catalase enhanced significantly in LTS as compared with those 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).

Plasma a-tocopherol and TBARs. There were no significant changes in a-tocopherol, an antioxidant vitamin, and TBARs, which is an index of lipid peroxidation, in STS. However, decrease in a-tocopherol and increase in TBARs levels were found statistically significant (p<0.05) in LTS. Additionally, to examine whether smoking period is effective 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 (p<0.05) (table-2).

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

<table>
<thead>
<tr>
<th></th>
<th>Young Non-smokers (n=8)</th>
<th>Short term smokers (n=8)</th>
<th>Old Non-smokers (n=8)</th>
<th>Long term smokers (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD(IU.gHb⁻¹)</td>
<td>1734.00</td>
<td>1751.50</td>
<td>1705.00</td>
<td>1751.50</td>
</tr>
<tr>
<td>Catalase(k.gHb⁻¹)</td>
<td>1456.50</td>
<td>1493.50</td>
<td>1437.50</td>
<td>1494.00</td>
</tr>
<tr>
<td>T.GSH (mg.gHb⁻¹)</td>
<td>1.10</td>
<td>1.10</td>
<td>0.95</td>
<td>0.76</td>
</tr>
<tr>
<td>GSH(mg.gHb⁻¹)</td>
<td>0.97</td>
<td>0.77</td>
<td>0.83</td>
<td>0.51</td>
</tr>
<tr>
<td>GSSG(mg.gHb⁻¹)</td>
<td>0.11</td>
<td>0.20</td>
<td>0.09</td>
<td>0.23</td>
</tr>
<tr>
<td>TBARs(nMol.ml⁻¹)</td>
<td>1.81</td>
<td>1.91</td>
<td>1.82</td>
<td>2.08</td>
</tr>
<tr>
<td>Vit.E (mmol/L)</td>
<td>14.66</td>
<td>13.75</td>
<td>14.05</td>
<td>11.07</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01; ; The statistical comparlslon Is between Current smokers and their control values.

DISCUSSION

Our findings related to 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 not 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 O₂ metabolites, such as H₂O₂ or H₂O₂-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 that 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 concentration of RBC unchanged in passive smokers whereas in our study GSH and vitamin E concentrations decreased while catalase activity increased in LTS. Possible explanation for these differences in results may be due to, the fact that the subjects, employed in their study, were young and passive
It was reported that erythrocytes from healthy cigarette smokers contain more glutathione and catalase, and protect lung endothelial cells from \textit{H2O2} better than do erythrocytes from age-and gender-matched non-smokers (7). In the present study, catalase activity was similar compared with Toth et al (7) but, glutathione level was different in cigarette smokers. We studied on two smoker groups separately and observed the changes in LTS. Thus, conflicting results with those of Toth et al (7) obtained from glutathione may be due to differences in the physical characteristics and smoking period of the subjects. McCusker and Hoidal (18) indicated that activities of SOD and catalase from AMs of cigarette smokers and smoke exposed hamsters were twice that found in control subjects. Increases in erythrocytes 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 to say that our results show similarities with those from McCusker and Hoidal (18).

In addition, Abou-Seif (8) observed that lower plasma vitamin E levels and higher erythrocyte SOD and catalase activities in cigarette smokers in compared with nonsmokers. This results are in good consistent with our data obtained from LTS. However, another study pointed out increased production of oxygen radical species, and decrease 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 through increased oxidant stress (4).

To investigate whether cigarette smoking would affect serum \textit{a-tocopherol} concentration and TBARs level as an index of lipid peroxidation, we evaluated these parameters in smokers and non-smokers. Elevated serum TBARs level and reduced \textit{a-tocopherol} concentration suggest that lipid peroxidation is enhanced in LTS. Similar results were demonstrated by Jendryczko et al (6) in passive smokers.

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 enzymatic and non-enzymatic antioxidant defense system of elderly smokers.
SİGARA İÇİMİNİN KAN ANTIOKSIDAN DÜZEYİ VE LİPID PEROKSİDASYONUNA ETKİLERİ

Bu çalışma sigara içiminin kan antioksidan düzeyi ve lipid peroksidasyonu üzerine etkisini araştırmak amacıyla yapıldı. Bunun için sağlık erkeklerden, 16 sigara tiryakisi ve sigara içmeyen 16 kontrol birey çalışmaya alındı. Sigara içen bireyler, kısa süre iççiler (short term smokers-STS) ve uzun süre iççiler (long term smokers-LTS) olmak üzere iki grupta incelendi. Her iki sigara içen grubun benzer yaş ve fiziksel özelliklerine sahip sigara içmeyen birer kontrol grubu oluşturuldu.

Uzun ve kısa süreli iççiler kendi kontrol grubu değerleri ile karşılaştırıldığında;

1- Uzun süre sigara içen bireylerde süperoksid dismutaz (SOD) ve katalaz aktivitesi önemli ölçüde artış gösterirken (p<0.05), kısa süre iççilerde enzim aktiviteleri değişmedi.

2- Kısa süre sigara kullanan bireylerin total ve reduced Glutatyon (GSH) düzeyleri kontrol değerlerle benzerlik gösterirken, oksidize glutatyon (GSSG) miktarı sigara iççilerinde anlamlı ölçüde arttı (p<0.01).

3- Uzun süre sigara içici olma değerlendirilen GSH azalırken (p<0.01) GSSG anlamlı artış gösterdi (p<0.01) fakat total GSH değişmedi.

4- Bulgularımızda göre kısa süre sigara içimi Vitamin E düzeyini etkilemedi. Ancak uzun süre iççilerde bu değer anlamlı ölçüde azalma gösterdi (P<0.05).

5- Lipt peroksidasyonunun bir göstergesi olan serum Thiobarbituric acide reactive substance (TBADs) düzeyinde uzun süre iççilerde önemli artışlar gözlenirken kısa süre iççilerde değişmedi.

Sigara içen bireyler kendi aralarında karşılaştırıldığında; uzun süre sigara içen bireylerde total GSH, reduced GSH ve Vitamin E düzeylerinde anlamlı azalmalar görüldü. Ancak ölçülen diğer parametrelerde anlamlı fark saptanamadı.

Sonuç olarak; uzun süre sigara içimi, bireylerin enzimatik ve non-enzimatik antioksidan savunma sistemlerini zayıflattığı ve bu zayıflığı, uzun süre sigara kullanımının organizmada yol açtığı oksidatif stres ile bağlı olduğu söylenebilir.

Anahtar Kelimeler: Oksidatif stress, Antioksidanlar, TBARs.

REFERENCES


