Analysis of Phospholipids Composition of Erythrocytes Isolated from Cigarette Smokers’ Blood

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Abstract

The effects of cigarette smoke on phospholipids composition of human red blood cells (RBCs) were assessed. Results of phospholipids composition of RBCs showed that, the quantities but not qualities was changed. For example, phosphatidylcholine, phosphatidylethanolamine, sphingomyeline, phosphatidylserine were decreased, while cardiolipin, lysophosphatidylecholine, and phosphatidylinositol were increased.

The cigarette smoke also induce a significant increase in cholesterol and triglycerides levels in serum of current smokers (240±20 and 155±10, respectively) than those from non-smokers (145±20, 80±10, respectively). Serum levels of HDL-cholesterol in smokers is decreased (35±5.0) when compared to the nonsmokers (55±8.0).

The results indicate that, cigarette smoking has very serious effects on peroxidation of RBCs phospholipids, and these effects may be due to the production of free radical species.

Key Words: Phospholipids, Thin Layer Chromatography, Cholesterol, Triglycerides, Erythrocytes, Lipid peroxidation.

Introduction

Cigarette smoking has been implicated in the etiology of respiratory diseases, Cancer, and atherosclerosis (1,2). Tobacco smoke contains large numbers of free radical species that are capable of initiating or promoting oxidative injury (3). Reactive oxygen species may also be generated in smokers by phagocytes that are activated in response to pulmonary inflammation (4,5). Several studies have suggested that oxidative injury may play a seminal role in mediating the health risks associated with cigarette smoking (6). Cigarette smokers have higher lipid peroxidation products in their blood than nonsmokers (7,8).

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Free radicals can attack the polyunsaturated fatty acids side chain of membrane lipids to initiate the process of lipid peroxidation (9,10). This process results in fragmentation of the lipid membranes and the formation of new reactive products called lipid peroxides, which are powerful inhibitors for many cellular enzymes, and can damage proteins and membranes (11), also may cause nucleic acid damage and protein sulfhydryl oxidation, which leads to intracellular enzyme inactivation (12).

Protection from sulfhydryl oxidation is felt to be provided in the cell by the tripeptide glutathione (GSH). Other protective mechanisms that have evolved include specific intracellular enzymatic defenses that can eliminate free radicals once they are formed. These oxidant defenses include the enzymes superoxide dismutase (SOD), catalase, and GSH-Px, also enzymatic defenses against oxidant radicals can be aided by nonspecific free radical scavengers such as α-tocopherol (vitamin E) (13,14), and also vitamin C, vitamin A, and selenium (15,16,17).

The mechanisms by which cigarette smoke affect the human health is not fully understood, but it may be due to promoting platelets aggregation, enhancing the production of free radicals, and changing in the elasticity of the blood vessels wall.

The aim of our study is to assess the effects of the production of free radical species by cigarette smoke on lipid peroxidation of RBCs and on the changes of biochemical and hematological parameters in cigarette smokers.

Materials and Methods

Blood samples were drawn from 35 male current smokers (smoke more than 20 cigarettes per day) (28 ± 5 yr. of age), and from 29 male nonsmokers (25 ± 4 yr. of age) and were anticoagulated with ethylenediamine tetra-acetic acid (EDTA). On the basis of the questionnaire, we chose volunteer who has no medication, his weight to height is nearly related, not alcohol drinker, and he is cigarette smoker not less than eight years.

Portion of EDTA blood sample was transferred to Coulter Counter apparatus for estimation of some hematological parameters, and the other portion was transferred to a centrifuge tube and centrifuged for 10 minutes at 3000 rpm to precipitate the erythrocytes. The supernatant liquid was drowning off; and the erythrocyte residue were washed with three times in normal saline solution. The lipids of erythrocytes was extracted by the method of Bligh (18), using a mixture of chloroform: methanol (2:1 v/v), followed by centrifugation at 3000 rpm for 20
minutes. The upper layer was discarded, and the lower layer of chloroform was evaporated using rotary evaporator.

Analysis of phospholipids, was done by using thin layer chromatography (TLC) with Silica gel plates with different systems: A- chloroform: methanol: H₂O (65:25:4 v/v); B- chloroform: methanol: 8% CaCl₂ (60:35: 8 v/v); C- chloroform: methanol: Acetone (8:1:1). The materials on plate were visualized by using iodine vapor (for general lipids) and ammonium molybdate solution (specific for phospholipids). The quality of phospholipids spots on plates was determined using standard phospholipids: PC, PE, PG, PS, PI, cardioliipin (diphosphoglycerate) (DPG), and Sphingomyeline. The quantity of phospholipids content was measured by densitometer.

To give further information about the effects of cigarette smoke on some metabolic parameters in these volunteers, we estimated the serum concentrations of cholesterol, triglycerides, and HDL-cholesterol; blood was drawn (vein puncture) from the above volunteers (fasting for at least 12 hours); centrifuged at 3000 rpm for 15 minutes, and then the serum was taken off, and used for estimation of the concentrations of cholesterol, triglycerides and HDL-cholesterol by using specific kit for each.

Reagents

Cholesterol Kit. (Enzymatic colorimetric test (CHOD- PAP)), Triglycerides Kit (Enzymatic- colorimetric test (GPO-PAP)), EDTA tubes, Coulter Counter model-T-860 USA (for the estimation of some hematological parameters). HDL- Cholesterol Kit (BioMerieux Sa-France 64531), Chloroform, Methanol, and other chemicals all of AR grade, Silica gel plates 60 F (Germany), Ammonium molybdate, Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserin (PS), Phosphatidylinositol (PI) and Sphingomyeline (SM) all from Kharkove Factory (Okramia), Densitometer 300 (Biomid-France), Rotary evaporator (vv 2000.D 8420 Kelheim, Germany).

Statistical Analysis:

The data are given as mean ± SD. Significance of differences was analyzed using Student’s t test; probability values of < 0.05 were considered significant.
Results and Discussion:

Analysis of phospholipids content of erythrocytes are shown in table (1). It was indicated that the types of the erythrocyte's phospholipids that are extracted from these smokers and nonsmokers did not changed, but the quantities of these types of lipids were changed, for example, in current smokers the following phospholipids are decreased: PC, PG, PS, PE and SM, while PL, DPG and LPC are increased, this suggests that cigarette smoking enhance lipid peroxidation, and then the formation of lipid peroxides and lyso compounds that affect the phospholipids content of erythrocytes, resulting in oxidation of phospholipids which lead to the change in their contents, as some of them were converted to other types and lyso compounds. The peroxidation of lipids will enhance the tissues injury leading to the changes in the erythrocytes shape and function. The increase of 2,3-DPG also affects the hemoglobin affinity for O₂ and thus shifts the hemoglobin-oxygen dissociation curve to the right.

Estimation of serum levels of cholesterol, triglycerides, and HDL-cholesterol are shown in table (2), which shows that there is an increase in the levels of cholesterol and triglycerides in serum of current smokers (240±20, 155±10, respectively), when compared to healthy nonsmokers (145±20, 80±10, respectively) which suggest that these volunteers may be in high risk at heart attack or other coronary heart diseases.

The HDL-cholesterol concentrations of current smokers were decreased (35±5.0)( see table 2), when compared to healthy nonsmokers (55±8.0). This decrease in HDL-cholesterol levels is associated with a great risk for many heart diseases. Because the HDL acts as a scavenger of cholesterol and prevent their precipitation on the blood vessels. Therefore increasing the levels of HDL-cholesterol over 40 mg/dl will markedly lower the risk for heart attack or other heart diseases even if the total cholesterol concentration over 200 mg/dl.

Table (3) shows values of different hematological tests in non-smokers (control), and smokers volunteers and the normal values. There is a slight increase or change in WBC count, MCH, and MCHC, this change is similar to what has been written in literature(19), that cigarette smoking is consider as one factor for causing of secondary polycythemia vera where the MCH, MCHC is increased.
Conclusion:

Our results indicated that, the change in phospholipid composition of erythrocytes, and high serum levels of cholesterol, triglycerides, and the low levels of HDL-cholesterol in these young smokers are a significant factors illustrating that these individuals are at high risk from many disorders mainly heart diseases (atherosclerosis) and lung diseases. These effects which are due to the presence of toxic compounds and materials in cigarette smoke itself that may be directly or indirectly affect human health. The presence of some toxic compounds or products will enhance the production of free radicals that result in lipid peroxidation, that lead to the formation of lipid peroxide, which result in many changes as injury of some tissues specially lung tissues, and alteration in the RBCs membranes that affect the quantity of some phospholipids and may be even to the shape of RBCs. Other effects as promoting the aggregation of platelets as a result of the effects of nicotine, and also it decreases the antioxidants in the cell or in the serum, that enhance their effects on the peroxidation of lipids.

Additional studies are required to describe the effects of cigarette smoking on other type of cells, other hematological parameters, and on the state of antioxidant system in serum and RBCs.

References


تحليل الليميبادات الفوسفورية لخلايا الدم الحمراء المعزولة من دم المدخنين

مختصر

لقد تمت دراسة تأثير التدخين على مكونات الليميبادات الفوسفورية الموجودة في خلايا الدم الحمراء. وقد بينت نتائج دراسة مكونات الليميبادات الفوسفورية المستخلصة من خلايا الدم الحمراء لهؤلاء المدخنين أن نوعية هذه الليميبادات لم تتفاوت، مقارنة مع مثيلاتها المستخلصة من خلايا الدم الحمراء لغير المدخنين، بينما كمية (نسبة) هذه الليميبادات قد تغيرت، فعلى سبيل المثال، الفوسفاتيد كولين، الفوسفاتيد إيثانول أمين، الفوسفاتيد سيرين، والسفونوميلين، قد انخفضت نسبتها، بينما الغليسبرول ثنائي الفوسفات، الليزو فوسفاتيد كولين، والفوسفاتيد أينوسيتول قد زادت نسبتها.

و للتدخين أثر واضح على زيادة مستويات الكوليسترول والغليسريدات الثلاثية في مصل الدم (240 ± 20 ، 150 ± 10 على التوالي)، مقارة مع غير المدخنين (145 ± 20، 80 ± 10 على التوالي). (و ان مستويات الكوليسترول المحمي في بلازم الدم لهؤلاء المدخنين قد انخفضت (135 ± 50 مقارنة مع غير المدخنين (50 ± 8، 0) .

يتضح من هذه الدراسة أن للتدخين أثر واضح في اكساء الليميبادات الفوسفورية المكونة لخلايا الدم الحمراء والتي تؤدي إلى تغير في تركيب هذه الخلايا مما يؤدي إلى خلل في وظيفتها.

**Table 1**

The percentage of the phospholipids composition of erythrocytes in nonsmokers and current smokers. LPC: Lysophosphatidylcholine; SM: Sphingomyelene; PS: Phosphatidylserine; PI: Phosphatidylinositol; PC: Phosphatidycholine; PG: Phosphatidylglycerol; PE: Phosphatidylethanolamine; DPG: Diphosphoglycerate or cardiolipin. # n = 35, * n = 29, P < 0.05.

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Control (nonsmokers)</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPC</td>
<td>0.8±0.1</td>
<td>3.5±0.8</td>
</tr>
<tr>
<td>SM</td>
<td>18.0±1.2</td>
<td>17.6±0.5</td>
</tr>
<tr>
<td>PS</td>
<td>12.0±0.8</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>PI</td>
<td>6.0±0.7</td>
<td>9.7±1.0</td>
</tr>
<tr>
<td>PC</td>
<td>25.0±1.3</td>
<td>22.1±1.1</td>
</tr>
<tr>
<td>PG</td>
<td>7.0±0.8</td>
<td>5.0±0.8</td>
</tr>
<tr>
<td>PE</td>
<td>25.0±1.5</td>
<td>19.5±1.4</td>
</tr>
<tr>
<td>DPG</td>
<td>7.0±1.0</td>
<td>12.0±0.9</td>
</tr>
</tbody>
</table>
Table 2
Serum concentrations (mg/dl) of cholesterol, triglycerides, and HDL-cholesterol in current smokers, nonsmokers (control) volunteers, and normal values, \#n = 35;

* n = 29. P < 0.05.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>*Nonsmoker</th>
<th>#Smokers</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>145±20</td>
<td>240±20</td>
<td>140±20</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>80±10</td>
<td>155±10</td>
<td>60±6.0</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>55±8.0</td>
<td>35±5.0</td>
<td>&gt;40.0</td>
</tr>
</tbody>
</table>

Table 3
Values of some hematological parameters in current smokers, nonsmoker volunteers, and normal values. P < 0.05.

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Normal values</th>
<th>Nonsmokers n=29</th>
<th>Smokers n=35</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count ((10^{12}/l))</td>
<td>4.7 - 6.1</td>
<td>5.0±0.5</td>
<td>6.5±0.5</td>
</tr>
<tr>
<td>WBC count ((10^{9}/l))</td>
<td>4.8 - 0.8</td>
<td>6.5±3.0</td>
<td>10±1.5</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14 - 16</td>
<td>15.3±1.0</td>
<td>16±1.0</td>
</tr>
<tr>
<td>PCV(%)</td>
<td>42 - 52</td>
<td>46±1.5</td>
<td>47±2.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>80-94</td>
<td>84±2.0</td>
<td>85±3.0</td>
</tr>
<tr>
<td>MCH (pg.)</td>
<td>27 - 31</td>
<td>29±0.8</td>
<td>31.5±0.3</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33 - 37</td>
<td>33±0.8</td>
<td>36±0.25</td>
</tr>
</tbody>
</table>