Effect of Smoking on the Serum Concentration of Erythropoietin and Granulocyte-Colony Stimulating Factor

Noboru Tanabe, Kazunori Ohnishi*, Hisao Fukui** and Ryuzo Ohno*

Smoking is the most common cause of secondary polycythemia and may induce leukocytosis. We studied the relationship between hematopoietic growth factors and erythrocytosis and leukocytosis. Two sets of healthy male volunteers, consisting of 177 and 202 (age: 19–59 years) were each divided into four groups according to whether or not they smoked at least one package daily and their leukocyte count. Serum erythropoietin (Epo) concentration and granulocyte-colony stimulating factor (G-CSF) concentration were measured in the 177 and 202 volunteers, respectively. The mean serum Epo concentration was lower in smokers than in nonsmokers (p=0.01 in the subjects without leukocytosis and p=0.107 in those with leukocytosis, respectively). After 3 smokers stopped smoking, the Epo concentration increased 2 weeks later, and remained constant for 20 weeks. Smokers tended to have a higher mean serum G-CSF concentration than nonsmokers in the subjects without leukocytosis. Neither Epo nor G-CSF is the main etiology of smokers’ polycythemia, and Epo production may be down-regulated by an elevated red-cell volume.

Key words: granulocyte-colony stimulating factor (G-CSF), polycythemia, leukocytosis

Introduction

Cigarette smoking is one of the largest public health problems in the world. Smokers are at greater risk for developing cancer, stroke and atherosclerosis. The risk of thromboembolic events, including brain infarction and myocardial infarction, are the most important disease entities. Thromboembolic episodes have a variety of etiologies. Virchow identified three major risk factors for thrombosis: blood quality, state of the vasculature, and blood flow (1). Polycythemia increases turbidity and induces rheological changes in blood. Polycythemia in smokers is thought to be due to the continuous exposure to carbon monoxide in tobacco smoke, where excessive and continuous exposure to carbon monoxide in smoke produces hypoxia (2). However, Miller et al reported no detectable differences in the erythropoietin (Epo) concentration between smokers and nonsmokers (3).

Furthermore, smokers often are found to have incidental leukocytosis (4). In most cases, the leukocyte count is normal on repeat examination, but a small percentage of people demonstrate persistent leukocytosis on serial examinations and are referred to a hematologist. Such patients are rarely found to be suffering from a myeloproliferative disorder. However, various studies, including the Edinburgh Artery Study (5) and the NHANES I Study (6) recently established leukocytosis as a risk factor in coronary artery disease. Therefore, ruling out myeloproliferative disease alone is not enough in patients with persistent leukocytosis. If the most important coronary risk factor in smokers is leukocytosis, the relationship between these two factors should be investigated more fully. As the sensitivity of the method of analysis had not been sufficient for detection of the normal granulocyte-colony stimulating factor (G-CSF) level at less than 30 pg/ml, there has been no report measuring the serum concentration of G-CSF in smokers. But a new method using chemiluminescent compounds has provided an extremely sensitive mode for detection (7).

In this study, we examined whether Epo is responsible for smokers’ polycythemia, and measured the serum concentration of G-CSF in smokers and determined the relationship between smoking and leukocytosis.
Serum Erythropoietin and G-CSF in Smokers

Materials and Methods

Serum Erythropoietin Concentration

Peripheral blood samples were obtained from 177 healthy male employees of a suburban car-parts factory (Nippon-Denso Co. Ltd., Kariya) during their routine annual health examination. Since the relationship between erythrocytosis, leukocytosis and the growth factors was not clear in smokers, subjects were separated into four groups according to smoking habit and leukocytosis as follows: group A (n=50); nonsmokers with a normal leukocyte count; group B (n=50), smokers (>20 cigarettes per day) with a normal leukocyte count; group C (n=50), smokers with leukocytosis (>9,000/μl with no identifiable etiology other than smoking); and group D (n=27), nonsmokers with leukocytosis. Blood cell counts, hemoglobin concentration and hematocrit were measured by Coulter Model S, and the Epo concentration was measured by radio-immunoassay (Erythropoietin RIA Chugai kit, Chugai Pharmaceutical Co., Ltd., Tokyo) (8). Three smokers stopped smoking (they declared cessation of smoking by themselves), and blood was obtained to measure blood counts and erythropoietin concentration 2, 4 and 20 weeks after the cessation of smoking. However, cessation of smoking was not confirmed by measuring carboxyhemoglobin but by their declaration.

Serum G-CSF Concentration

Four groups were created from another cohort of 202 randomly selected healthy male factory workers (Nippon-Denso Co. Ltd.), because no sensitive method for measuring G-CSF was available when the samples were collected from the first set of 177 volunteers to measure Epo. Group E (n=60), nonsmokers with a normal leukocyte count; group F (n=60), smokers (>20 cigarettes per day) with a normal leukocyte count; group G (n=60), smokers with leukocytosis (>9,000/μl on two sequential blood examinations with no identifiable etiology other than smoking); group H (n=22), nonsmokers with leukocytosis. The G-CSF concentration was measured by a highly sensitive chemiluminescent immunoassay (Chugai Pharmaceutical Co., Ltd., Tokyo) (7). Data were analyzed using the Student-t test for the normal distribution or the Mann-Whitney's U test when the data did not show the normal distribution. Informed consent was obtained from each subject before phlebotomy.

Table 1. Serum Erythropoietin Concentration and Hematologic Findings in Smokers and Nonsmokers

<table>
<thead>
<tr>
<th>Group</th>
<th>Smoking</th>
<th>Leukocytosis</th>
<th>No.</th>
<th>Epo* (mU/ml)</th>
<th>Leukocyte (μl)</th>
<th>Erythrocyte (μl)</th>
<th>Hematocrit** (%)</th>
<th>Hemoglobin*** (g/dl)</th>
<th>Platelet (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No</td>
<td>No</td>
<td>50</td>
<td>18.4 ± 5.4</td>
<td>6,120 ± 1,300</td>
<td>482 ± 29</td>
<td>43.9 ± 2.2</td>
<td>14.9 ± 0.8</td>
<td>25.5 ± 4.1</td>
</tr>
<tr>
<td>B</td>
<td>Yes</td>
<td>No</td>
<td>50</td>
<td>14.9 ± 6.7</td>
<td>6,720 ± 1,600</td>
<td>476 ± 36</td>
<td>44.3 ± 6.1</td>
<td>15.1 ± 1.0</td>
<td>25.3 ± 4.6</td>
</tr>
<tr>
<td>C</td>
<td>Yes</td>
<td>Yes</td>
<td>50</td>
<td>17.3 ± 4.1</td>
<td>11,000 ± 2,300</td>
<td>486 ± 37</td>
<td>45.4 ± 2.7</td>
<td>15.7 ± 1.0</td>
<td>28.2 ± 5.1</td>
</tr>
<tr>
<td>D</td>
<td>No</td>
<td>Yes</td>
<td>27</td>
<td>19.7 ± 4.4</td>
<td>8,890 ± 2,500</td>
<td>477 ± 56</td>
<td>42.8 ± 5.3</td>
<td>14.7 ± 1.8</td>
<td>31.8 ± 6.4</td>
</tr>
</tbody>
</table>

*Comparison of serum erythropoietin (Epo) by Mann-Whitney’s U test; A vs B: p=0.01, C vs D: p=0.107, A vs D: p=0.238, B vs C: p=0.003. **Comparison of Ht by Student’s t-test; A vs B: p=0.431, C vs D: p=0.002, A vs D: p=0.226, B vs C: p=0.043. ***Comparison of Hb by Student’s t-test; A vs B: 0.324, C vs D: p=0.007, A vs D: p=0.359, B vs C: p=0.004.

Results

Serum Erythropoietin Concentration in Smokers

Table 1 summarizes the serum Epo and the hematologic features in smokers and nonsmokers. In the subjects without leukocytosis, Epo concentration was significantly lower in smokers (group B) than in nonsmokers (group A) (p=0.01 by the Mann-Whitney’s U test), but there were no differences in hemoglobin concentration and hematocrit between them. In the subjects with leukocytosis, Epo concentration tended to be lower in smokers (group C) than in nonsmokers (group D) (p=0.107 by the Mann-Whitney’s U test), and hemoglobin concentration and hematocrit were significantly higher in smokers than in nonsmokers. In smokers, Epo concentration, hemoglobin concentration and hematocrit were significantly higher in the subjects with leukocytosis (group C) than in those without leukocytosis (group B). In nonsmokers, Epo concentration, hemoglobin concentration and hematocrit except platelet count showed no differences between the subjects with leukocytosis (group D) and those without leukocytosis (group A).

In this study, there were few subjects with a high hemoglobin

Table 2. Serum Erythropoietin Concentration According to the Hemoglobin Concentration

<table>
<thead>
<tr>
<th>Hemoglobin* (g/dl)</th>
<th>No.</th>
<th>Epo (Mean ± SD) (mU/ml)</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Low</td>
<td>147</td>
<td>17.0 ± 5.7</td>
<td>0.04</td>
</tr>
<tr>
<td>High</td>
<td>30</td>
<td>18.8 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>Nonsmokers Low</td>
<td>70</td>
<td>18.6 ± 5.1</td>
<td>0.109</td>
</tr>
<tr>
<td>High</td>
<td>7</td>
<td>21.5 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Smokers Low</td>
<td>77</td>
<td>15.6 ± 5.8</td>
<td>0.023</td>
</tr>
<tr>
<td>High</td>
<td>23</td>
<td>17.9 ± 4.9</td>
<td></td>
</tr>
</tbody>
</table>

*Low: Hb <16 g/dl, High: Hb ≥16 g/dl. **Mann-Whitney’s U test.
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concentration of greater than 17 g/dl. Therefore we divided the subjects into two groups by the hemoglobin concentration of 16 g/dl and we compared the Epo concentration in them. As shown in Table 2, the Epo concentration was higher in the group with a high hemoglobin concentration of more than 16 g/dl than in the group with a low hemoglobin concentration of less than 16 g/dl in all subjects (p=0.04 by the Mann-Whitney's U test), in nonsmokers (p=0.109), or in smokers (p=0.023), respectively.

Figure 1 shows the changes in hematological parameters and the serum Epo concentration following cessation of smoking in three smokers. Two weeks after cessation of smoking, erythrocyte count, reticulocyte count, hematocrit, hemoglobin concentration and neutrophil count decreased. Four weeks after cessation of smoking, erythrocyte count, hematocrit and reticulocyte count returned to the levels of the smoking period, but the hemoglobin concentration and neutrophil count remained at the

Figure 1. Measurement of the serum erythropoietin and hematologic factors in three smokers before and after cessation of smoking.
Erythropoietin production may represent a balance between factors other than G-CSF. Therefore, we think that the secretion of Epo and an increase of the reticulocyte count.

Serum G-CSF Concentration in Smokers
In the subjects without leukocytosis, the mean G-CSF concentration tended to be higher in smokers (group F) than in nonsmokers (group E) (p=0.101 by Mann-Whitney’s U-test) (Table 3). However, there were no significant differences in G-CSF concentrations in the other combinations of groups. The G-CSF concentration varied widely, especially among subjects with leukocytosis (groups G and H). No significant correlation was detected between the leukocyte counts and G-CSF concentrations (data not shown).

Discussion
Numerous reports have documented that excessive carbon monoxide exposure caused by cigarette smoking produces polycythemia. Smith and Landaw have shown that smokers with elevated hematocrits have an increased blood carboxyhemoglobin, increased red-cell volume and reduced plasma volume (9). Galson et al have shown that the Epo gene is regulated by hypoxia-inducible cis-acting elements in the promoter and in the 3’ enhancer (10). Therefore, it was thought that smoking provokes tissue hypoxia and induces Epo production, leading to increased red cell volume. However, Miller et al found no difference in Epo concentrations between men and women, smokers and nonsmokers, or different age groups (3). They also failed to detect differences in the Epo concentration when the carboxyhemoglobin concentration changed following the cessation of smoking. In the present study, smokers had a significantly lower serum Epo concentration than nonsmokers, although their hemoglobin concentration and hematocrit were high. However, smokers with the high hemoglobin concentration had a significantly higher serum Epo concentration than smokers with the low hemoglobin concentration. When smokers stopped smoking, the hematocrit and hemoglobin concentration decreased while the serum erythropoietin concentration increased. It is likely that smoking induced hemconcentration initially and that serum Epo concentration was then down-regulated once the red-cell volume increased.

Erythropoietin production may represent a balance between stimulation by hypoxia and the negative feedback by a rising red-cell volume. The cessation of smoking induced a cascade, leading to recovery of the circulating plasma volume, a relative decrease of the hemoglobin and hematocrit, increased production of Epo and an increase of the reticulocyte count.

Measurement of the blood G-CSF concentration failed to show a significant difference between smokers and nonsmokers, and the values varied widely in the groups with leukocytosis. Multiple mechanisms may contribute to the neutrophilia, including the release of microbial and host tissue constituents, adrenergic stimulation, increased corticosteroid release and enhanced elaboration of cytokines that promote myelopoiesis. The kinetics of G-CSF might be more complicated than those of Epo which regulates erythropoietic production. The G-CSF is usually produced and works in the hematopoietic microenvironment, however, a very small quantity of G-CSF is released into circulation and it may reflect the production of G-CSF in the whole body. The effect of smoking on hemodynamics might also be mediated through biologically active substances, such as catecholamines and nicotine. Therefore, an interindividual variation in the G-CSF concentration may exist. Cox et al have shown that neutrophil accumulation in the respiratory tract occurs in a variety of inflammatory disorders, especially in those associated with cigarette smoking, and that bronchial epithelial cells markedly increase the survival of human neutrophils in vitro via the release of G-CSF and GM-CSF (11). The present results showed that the G-CSF concentration in smokers was not significantly higher than in nonsmokers. However, the G-CSF concentration should decrease via negative feedback, if smoking releases leukocytes via factors other than G-CSF. Therefore, we think that the secretion of G-CSF may be one of the etiologies that chronic abuse of smoking induces leukocytosis, even though it is not solely responsible. Recently, leukocytosis has been shown to be an independent risk factor for coronary heart disease (5, 12). Lowe et al have reported that elastase released from activated neutrophils may damage the vascular endothelium (5), while Klut et al have proven that tobacco smoking increases leukocyte activation in a rabbit model (13). Also, Lehr et al have reported that tobacco smoking promotes the adhesion of leukocytes to endothelium (14).

Serum Erythropoietin and G-CSF in Smokers

Table 3. Serum G-CSF Concentration and Hematologic Findings in Smokers and Nonsmokers

<table>
<thead>
<tr>
<th>Group</th>
<th>Smoking</th>
<th>Leukocytosis</th>
<th>No.</th>
<th>G-CSFa (pg/ml)</th>
<th>Leukocyte (/µl)</th>
<th>Erythrocyte (/µl)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/dl)</th>
<th>Platelet (M )</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>No</td>
<td>No</td>
<td>59</td>
<td>9.3 ± 4.0</td>
<td>5,748 ± 1,212</td>
<td>486 ± 26</td>
<td>44.4 ± 2.8</td>
<td>14.9 ± 8.9</td>
<td>26.2 ± 4.0</td>
</tr>
<tr>
<td>F</td>
<td>Yes</td>
<td>No</td>
<td>60</td>
<td>10.9 ± 5.3</td>
<td>6,472 ± 1,752</td>
<td>480 ± 39</td>
<td>44.5 ± 2.6</td>
<td>14.5 ± 2.8</td>
<td>24.5 ± 4.7</td>
</tr>
<tr>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>60</td>
<td>12.8 ± 10.8</td>
<td>10,375 ± 2,588</td>
<td>490 ± 38</td>
<td>43.8 ± 2.5</td>
<td>15.3 ± 0.9</td>
<td>27.3 ± 5.2</td>
</tr>
<tr>
<td>H</td>
<td>No</td>
<td>Yes</td>
<td>18</td>
<td>11.5 ± 12.2</td>
<td>9,278 ± 2,610</td>
<td>478 ± 50</td>
<td>43.2 ± 4.7</td>
<td>14.7 ± 1.6</td>
<td>31.1 ± 6.0</td>
</tr>
</tbody>
</table>

*aComparison of G-CSF by Mann-Whitney’s U-test; E vs F: p=0.101, G vs H: p=0.810, E vs H: p=0.716, F vs G: p=0.373.

Table 3. Serum G-CSF Concentration and Hematologic Findings in Smokers and Nonsmokers

Smoking decreased levels for 20 weeks after cessation of smoking. On the other hand, the serum Epo concentration increased during the first 2 weeks after cessation of smoking, and then stabilized in two subjects and continued to increase in the other subjects.
smokers’ leukocytosis to prevent ischemic heart disease.

References