Serum Thioredoxin and α-Tocopherol Concentrations in Patients With Major Risk Factors

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Background Oxidative stress, which is thought to be increased in subjects with various coronary risk factors, induces thioredoxin (TRX), a redox-active protein.

Methods and Results To determine whether oxidative stress is increased, serum concentrations of both TRX and α-tocopherol (vitamin E) were determined in 12 control subjects without any coronary risk factors (CONTROL), 6 current smokers (SMOKING), 19 hypertensive patients (HT), 7 hypercholesterolemic patients (HC) and 14 subjects with multiple risk factors (MULTIPLE). Patients with diabetes mellitus were not included. The serum TRX concentrations (mean±SD ng/ml) were significantly higher in SMOKING (41±10), HT (41±17), HC (48±15) and MULTIPLE (46±15) than in CONTROL (24±11). The serum α-tocopherol concentrations (mg/g lipids) were not significantly different among CONTROL (4.0±0.7), SMOKING (4.0±0.8), HT (4.1±0.6) and HC (4.2±0.6), although the concentration was significantly lower in MULTIPLE (3.3±0.7) than in any of the other study groups.

Conclusions SMOKING, HT, HC and MULTIPLE had significantly higher serum TRX concentrations than CONTROL, suggesting increased oxidative stress. MULTIPLE had a lower serum concentration of antioxidant α-tocopherol than any of the other study groups, suggesting impaired or exhausted defense against chronic oxidative stress in the presence of the multiple risk factors. (Circ J 2005; 69: 291–294)

Key Words: α-Tocopherol; Coronary risk factors; Oxidative stress; Thioredoxin

The important role of oxidative modification of lipoproteins has been implicated in the formation of the early lesions of atherosclerosis. We have reported that circulating plasma low-density lipoprotein (LDL) was highly susceptible to oxidative modification in patients with variant angina and vitamin E (α-tocopherol) deficiency. A major risk factor for coronary spasm is smoking, which generates a large number of oxidants or free radicals, and it has been also suggested that oxidative stress is increased in subjects with various other coronary risk factors such as hypertension and hypercholesterolemia, as well as diabetes mellitus.

Thioredoxin (TRX) was originally discovered in Escherichia coli as an electron donor to ribonucleotide reductase. It is a multifunctional redox-active protein and reduces protein-disulfides together with TRX reductase and NADPH. TRX is induced by many forms of oxidative stress and released from cells serum concentrations are elevated in patients infected with the human immunodeficiency virus or hepatitis C virus, and in those with heart failure or fulminant myocarditis and recently we reported that serum TRX concentrations were elevated in patients with coronary vasospasm.

To determine whether oxidative stress is increased in subjects with various coronary risk factors such as smoking, hypertension and hypercholesterolemia, the serum concentrations of both TRX and vitamin E (α-tocopherol) were determined and compared with those in control subjects.

Methods

Study Patients Six, consecutive apparently healthy current smokers (SMOKING), 19 patients with uncomplicated hypertension (HT) and 7 with uncomplicated hypercholesterolemia (HC: >230 mg/dl), as well as 14 patients with 2 or more risk factors of smoking, HT and HC (MULTIPLE) were selected. For the control group (CONTROL), 12 age- and sex-matched subjects (8 men, 4 women; mean age, 55±15 years) without any significant systemic disease who had negative results for a treadmill exercise stress test, were selected. Patients with diabetes mellitus or hemoglobin A1c >6.5% were not included in the study, nor were patients receiving antioxidants or lipid-lowering medication. The present investigation was conducted prior to patients receiving any drug therapy. Written informed consent was obtained from all the study patients, and the study protocol was approved by the institutional ethics committee.

Serum Analysis Venous samples were obtained after overnight fasting. The LDL-cholesterol concentration was calculated according to the Friedwald equation. Serum concentrations of

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TRX were determined by an enzyme-linked immunosorbent assay\(^{19,26}\) and those of \(\alpha\)-tocopherol were determined by high-performance liquid chromatography\(^2,3\).

**Assessment of Coronary Risk Factors**
If the patient had smoked more than 15 cigarettes/day over the preceding 20 years they were defined as a current smoker; HT was defined as a persistent resting blood pressure exceeding 140 (systolic) or 90 (diastolic) mmHg; body mass index (BMI) was calculated as weight (kg)/height (m)\(^2\).

**Statistical Analysis**
Values are presented as mean ± standard deviation. Comparisons of age and serum concentrations of lipids, TRX and \(\alpha\)-tocopherol among the study groups were performed with one-way ANOVA followed by the unpaired Student’s t-test. The level of significance was set as \(p<0.05\).

**Results**
The clinical characteristics of the study patients are summarized in Table 1. All the patients in MULTIPLE had HT. The BMI was significantly higher in both HT and MULTIPLE than in any of the other groups.

**Serum Lipid Concentrations**
The lipid profiles and the serum concentrations of TRX and \(\alpha\)-tocopherol are summarized in Table 2. The total cholesterol concentration was significantly higher in HC Table 1 Clinical Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>SMOKING</th>
<th>HT</th>
<th>HC</th>
<th>MULTIPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>12</td>
<td>6</td>
<td>19</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>M/F</td>
<td>8/4</td>
<td>5/1</td>
<td>10/9</td>
<td>3/4</td>
<td>9/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55(\pm)15</td>
<td>51(\pm)15</td>
<td>60(\pm)12</td>
<td>59(\pm)7</td>
<td>61(\pm)11</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>19 (100)</td>
<td>0 (0)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (100)</td>
<td>0 (0)</td>
<td>10 (71)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>22(\pm)3</td>
<td>22(\pm)3</td>
<td>24(\pm)*</td>
<td>22(\pm)1</td>
<td>24(\pm)4*</td>
</tr>
</tbody>
</table>

*p<0.05 vs CONTROL, SMOKING and HC.

HC, hypercholesterolemia; HT, hypertension.

Table 2 Serum Lipid Profiles of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>SMOKING</th>
<th>HT</th>
<th>HC</th>
<th>MULTIPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>12</td>
<td>6</td>
<td>19</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>192(\pm)26</td>
<td>180(\pm)30</td>
<td>208(\pm)20</td>
<td>245(\pm)9*</td>
<td>231(\pm)34*</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>113(\pm)27</td>
<td>97(\pm)41</td>
<td>124(\pm)25</td>
<td>164(\pm)14**</td>
<td>140(\pm)47**</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>77(\pm)28</td>
<td>100(\pm)54</td>
<td>94(\pm)39</td>
<td>110(\pm)26*</td>
<td>172(\pm)88**</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>64(\pm)16</td>
<td>63(\pm)19</td>
<td>65(\pm)21</td>
<td>59(\pm)11</td>
<td>57(\pm)15</td>
</tr>
<tr>
<td>Triolein (mg/ml)</td>
<td>24(\pm)11</td>
<td>41(\pm)10*</td>
<td>41(\pm)17*</td>
<td>48(\pm)15*</td>
<td>46(\pm)15*</td>
</tr>
<tr>
<td>Triolein (mg/g)</td>
<td>4.0(\pm)0.7</td>
<td>4.0(\pm)0.8</td>
<td>4.1(\pm)0.6</td>
<td>4.2(\pm)0.6</td>
<td>3.3(\pm)0.7**</td>
</tr>
</tbody>
</table>

*p<0.01 vs CONTROL, SMOKING and HT. **p<0.01 vs others, †p<0.01 vs CONTROL and SMOKING, ‡p<0.01 vs CONTROL, §p<0.05 vs others.

HT, hypertension; HC, hypercholesterolemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Fig 1. Comparison of the serum concentrations of thioredoxin (TRX). The concentration is significantly higher in the SMOKING, hypertension (HT), hypercholesterolemia (HC) and MULTIPLE groups than in the CONTROL group. The number of patients is shown in parentheses; mean ± standard deviation.
and MULTIPLE than in any of the other groups. The LDL-cholesterol concentration was significantly higher in HC than in any of the other groups, and also higher in MULTIPLE than in CONTROL and SMOKING. The triglyceride concentration was significantly higher in MULTIPLE than in any of the other groups and was significantly higher in HC than in CONTROL. Although the high-density lipoprotein (HDL)-cholesterol concentration was somewhat lower in HC and MULTIPLE, no significant difference was noted in its concentrations among the study groups.

Serum TRX Concentration

TRX concentrations in the study groups are plotted in Fig 1. The serum TRX concentrations (ng/ml) were significantly higher in SMOKING (41±10), HT (41±17), HC (48±15) and MULTIPLE (46±15) than in CONTROL (24±11). The TRX concentration was not significantly different among SMOKING, HT, HC and MULTIPLE.

Serum α-Tocopherol Concentration

The serum α-tocopherol concentrations (mg/g) corrected for total lipids (total cholesterol plus triglyceride) were comparable among CONTROL (4.0±0.7), SMOKING (4.0±0.8), HT (4.1±0.6) and HC (4.2±0.6) (Table 2, Fig 2). However, the concentration was significantly lower in MULTIPLE (3.3±0.7) than in any of the other groups.

The relationship between the serum concentrations of TRX and α-tocopherol was determined in all the study patients and no significant correlation (r=–0.05, NS) was observed.

Discussion

Serum TRX Concentration as a Measure of Oxidative Stress

TRX is a known cytokine-like factor with radical-scavenging functions and it has been suggested that the regulation of cellular reduction/oxidation (redox) by TRX plays an important role in signal transduction and cytoprotection against oxidative stress. Recently, Liu et al reported that TRX attenuated myosin-induced autoimmune myocarditis by suppressing chemokine expression and leukocyte chemotaxis in mice, suggesting it has anti-inflammatory effects. TRX is known to be induced by oxidative stress although the origins of serum TRX are varied. Serum concentrations have been reported to be elevated to approximately 30 ng/ml in patients with congestive heart failure, more than 100 ng/ml in a patient with fulminant myocarditis and also 64±44 ng/ml in patients with coronary spastic angina in the active stage. The present data on serum TRX concentrations, combined with its known properties, suggest that the serum concentration of TRX can be used as a generalized marker for oxidative stress and redox-equilibrium, although the correlation between serum TRX concentrations and the other markers for oxidative stress remains to be clarified and needs to be examined.

TRX in Subjects With Coronary Risk Factors

The present study has provided evidence of increased oxidative stress, as shown by elevated serum TRX concentrations, in patients with various risk factors including smoking, HT and HC, suggesting that these are causes of oxidative stress.

Cigarette smoke contains vast amounts of both carbon and oxygen-centered free radicals, which can directly initiate and propagate the process of lipid peroxidation. Several recent studies have shown that certain forms of genetic or acquired HT are associated with oxidative stress and that animals with those conditions respond favorably to antioxidant therapy. Oxidative stress may contribute to the generation and maintenance of HT via the inactivation of NO and the generation of vasoconstrictive substances. Endothelium-dependent vascular relaxation is also impaired in HC, and recent evidence suggests that increased degradation of endothelium-derived NO by the superoxide anion may be an important contributing factor in the progression of atherosclerosis.

In the present subjects with multiple risk factors, the serum α-tocopherol concentration was significantly lower than in the control subjects or those with a single risk factor, although the TRX concentration was not significantly different between the subjects with single and multiple risk factors. We surmise that there is exhaustion of endogenous antioxidant vitamin E because of augmented oxidative stress.
stress in subjects with multiple risk factors.

Although endogenous antioxidant \( \alpha \)-tocopherol appears to play an important role in preventing an excessive increase in oxidative stress, we did not find a significant correlation was observed between the serum concentrations of TRX and \( \alpha \)-tocopherol. The serum \( \alpha \)-tocopherol concentration may not be a direct marker for instantaneous oxidative stress, although chronically increased oxidative stress will exhaust the supply of antioxidant vitamins, resulting in lower serum \( \alpha \)-tocopherol concentrations. Recently we reported that the \( \alpha \)-tocopherol concentration was significantly diminished in subjects with various coronary risk factors including smoking, glucose intolerance, obesity and dyslipidemia in a population study using larger samples than in the present study. Multiple logistic regression analysis showed that both hypertriglyceridemia and hypoHDL-cholesterolemia were the significant risk factors for the lowest tertile of the \( \alpha \)-tocopherol concentration and other risk factors were not significant. It will need to be determined whether or not supplementation of \( \alpha \)-tocopherol will reduce the oxidative stress and result in a decrease of the TRX concentration.

Conclusions

Subjects with various coronary risk factors, including smoking, HT and HC, had higher TRX concentrations, suggesting increased oxidative stress. Subjects with multiple risk factors had lower serum concentrations of antioxidant \( \alpha \)-tocopherol, suggesting impaired or exhausted defense against chronic oxidative stress.

References


