Telomere Shortening of Peripheral Blood Mononuclear Cells in Coronary Disease Patients with Metabolic Disorders

Nobuya Obana, Sho Takagi, Yoshitaka Kinouchi, Yoshihisa Tokita*, Akihiro Sekikawa*, Seiichi Takahashi, Nobuo Hiwatashi, Shinichi Oikawa** and Tooru Shimosegawa

Abstract

Objective  Telomere shortening is correlated with cell turnover and aging, but it has been recently suggested to occur not only by aging but by several biochemical factors of metabolic disorders predisposing to atherosclerosis.

Patients and Methods  We compared telomere length of peripheral blood mononuclear cells of patients with the metabolic disorders, hypercholesterolemia (HC) and diabetes mellitus (DM), according to the presence or absence of coronary diseases.

Results  The results demonstrated that HC and/or DM patients with coronary diseases have significantly shorter telomere length than healthy controls (p=0.0014).

Conclusion  Telomere shortening may be involved in the mechanisms that promote coronary diseases under some circumstances of metabolic disorders.

Key words: telomere, coronary diseases, hypercholesterolemia, diabetes mellitus

Introduction

Telomeres are simple repetitive (TTAGGG)n-sequences located at the ends of chromosomes. They are thought to stabilize chromosomes and prevent them from end-to-end fusions or exonucleolytic degradations (1, 2). Because of an incapability of DNA polymerases to replicate the very ends of linear DNA, telomeres cannot be replicated completely by this enzyme, which results in a gradual shortening of telomere length with an increasing number of cell divisions and aging (3, 4). It is well known that there is a linear negative correlation between age and telomere length of peripheral blood mononuclear cells (5). Recent studies have shown that telomere shortening can be a biomarker of cell senescence and has been discussed on the pathologic relation to various diseases, which include atherosclerosis (6–10).

Metabolic disorders such as hypercholesterolemia (HC) or diabetes mellitus (DM) are causally related to atherosclerosis as has been reported by many previous studies. Recently, it has been emphasized that several biochemical factors such as oxygen radicals and elevated plasma homocysteine, which are overproduced in some metabolic disorders, are responsible for DNA damage and telomere shortening, and induce atherosclerosis (11–14). Therefore, in the present study, we examined telomere length of peripheral blood mononuclear cells of patients with HC and/or DM, and studied the relation to coronary diseases (CD) to clarify whether or not telomere shortening takes part in the development of atherosclerosis in such metabolic disorders.

For editorial comment, see p 135.

Methods

Samples

This study included 91 patients with HC and 84 patients with DM, of whom 35 had both diseases. Thirty healthy volunteers were enrolled as controls, who were older than 50 years of age to adjust the mean age of controls to that of patients' group. The criteria for the diagnosis of HC and DM were as follows: serum cholesterol levels ≥ 220 mg/dl or under lipid-lowering medication for HC, and fasting blood glucose levels ≥ 126 mg/dl on the new criteria or under treatment with per oral antidiabetic medicines for DM. Patients were evaluated for the presence of CD by electrocardiography or coronary angiography. These patients are classi-

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Telomere Shortening in Coronary Disease Patients

Table 1. Telomere Length Analysis Among 6 Subgroups Classified According to the Presence or Absence of HC and/or DM and CD

<table>
<thead>
<tr>
<th>Metabolic disorder</th>
<th>N</th>
<th>Age (years)</th>
<th>TRF (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD (+)</td>
<td>78</td>
<td>66±7.8</td>
<td>6.1±0.9*</td>
</tr>
<tr>
<td>HC (+) DM (+)</td>
<td>24</td>
<td>66±7.3</td>
<td>6.2±0.6</td>
</tr>
<tr>
<td>HC (+) DM (-)</td>
<td>19</td>
<td>66±9.4</td>
<td>5.9±1.2**</td>
</tr>
<tr>
<td>HC (-) DM (+)</td>
<td>35</td>
<td>66±7.5</td>
<td>6.2±0.7***</td>
</tr>
<tr>
<td>CD (-)</td>
<td>62</td>
<td>64±7.1</td>
<td>6.4±0.8</td>
</tr>
<tr>
<td>HC (+) DM (+)</td>
<td>11</td>
<td>64±5.4</td>
<td>6.6±0.7</td>
</tr>
<tr>
<td>HC (+) DM (-)</td>
<td>37</td>
<td>63±7.7</td>
<td>6.4±0.7</td>
</tr>
<tr>
<td>HC (-) DM (+)</td>
<td>14</td>
<td>66±6.8</td>
<td>6.4±0.9</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>64±6.4</td>
<td>6.9±1.5</td>
</tr>
</tbody>
</table>

TRF was significantly shorter in CD (+) group than Control (p=0.0014)*, CD (+) HC (+) DM (-) group than Control (p=0.021)**, and CD (+) HC (-) DM (+) group than Control (p=0.036)***.

Table 2. Clinical Characteristics of the Patients Classified According to the Presence or Absence of CD and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>CD (+)</th>
<th>CD (-)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>78</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66±7.8</td>
<td>64±7.1</td>
<td>64±6.4</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>50/28</td>
<td>42/20</td>
<td>22/8</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>44</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25±2.6</td>
<td>24±2.4</td>
<td>22±1.6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143±11.9</td>
<td>134±8.2</td>
<td>123±10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>88±9.5</td>
<td>80±11</td>
<td>74±7.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>227±31</td>
<td>231±29</td>
<td>184±21</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>135±23.4*</td>
<td>117±21</td>
<td>101±9</td>
</tr>
</tbody>
</table>

CD (+) means patient with coronary disease and CD (-) means without. *The value of serum glucose was higher in CD (+) group than CD (-) one (p<0.0001).

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TRF in the same sample. The interassay coefficient of variance was considered to be 2.1%.

Statistical analysis

All data are expressed as mean±SD. Parametric analysis of variance (ANOVA) was used to assess the differences of the mean TRF lengths among three groups, CD (+), CD (−) patients and controls. If the ANOVA revealed significant differences, a contrast analysis was performed using Scheffe’s multiple comparison test. The differences of clinical characteristics between CD (+) and CD (−) groups were assessed by unpaired t-test. Significant differences were defined by p values of < 0.05.

Results

First we supposed that patients with metabolic disorders had a shorter telomere length than controls, so we compared the telomere length of the following four groups: DM (−) HC (+) group, DM (+) HC (−) group, DM (+) HC (+) group and control. But we found no significant difference of telomere length among the four groups (data not shown). Next we classified the patients into two groups: CD (+) group and CD (−) group. As a result, CD (+) group had a significantly shorter telomere length compared to control, and in the CD (−) group, telomere length was shorter than control, but not significant. (Table 1)

Clinical characteristics of the subjects are shown in Table 2. There were no significant differences in clinical data between CD (+) and CD (−) groups except for the serum glucose level. The serum glucose level of CD (+) group was significantly higher than that of CD (−) group. The presence of DM in the CD (+) group (59 DM patients of 78 cases; 75.6%) was a higher percentage than that in the CD (−) group (25 DM patients of 62 cases; 40.3%), and the blood glucose control of DM in CD (+) group (mean fasting blood glucose = 145 mg/dl) was not as good as compared to DM in CD (−) group (mean fasting blood glucose = 129 mg/dl). These might be the reasons as to why there was a significance observed in the serum blood glucose level. Lipid levels were higher in both patient groups than in controls, but there was no significant difference between CD (+) and CD (−) group.

A representative example of Southern blot analysis of telomere length of peripheral blood mononuclear cells is shown in Fig. 1. In this figure, the CD (+) patient with HC (lane 2) shows a significantly shorter telomere than that of other CD (−) patients and control.

Telomere length of each group is shown in Table 1. CD (+) group had a significantly shorter telomere length compared to controls. In CD (−) group, the telomere length was shorter than control, but not significant.

We also studied the effect of the combination of metabolic diseases on the length of telomere as shown in Table 1. Each subgroup of CD (+) group had a shorter telomere than the CD (−) group and significantly shorter than control. Each subgroup of CD (−) group had a shorter telomere length than the control group, but not significant.

Discussion

In the present study, we demonstrated that the telomere length of peripheral blood mononuclear cells was significantly shorter in patients with CD. It is reported that telomere shortening occurs in correlation with cell turnover and aging. In addition, the telomere length of peripheral blood mononuclear cells has been reported to decrease gradually with aging, and change in parallel with that of coronary arterial endothelial cells, which may be prone to more rapid shortening than other endothelial cells because of their high turnover rate due to greater blood turbulence (5, 15, 16). In spite of the importance of telomere length in cell physiology, its regulation remains largely unknown. Al-

![Figure 1. A representative southern blot analysis of telomere length; lane1: 7.16 kb band seen in peripheral blood mononuclear cells (PBMC) of a healthy control aged 61 years, lane 2: 5.72 kb band seen in PBMC of a HC patient complicated with CD aged 65 years, lane 3: 6.56 kb band seen in PBMC of a HC patient not complicated with CD aged 60 years, lane 4: 7.25 kb band seen in PBMC of a DM patient not complicated with CD aged 63 years, lane 5: 6.84 kb band seen in PBMC of a HC and DM patient not complicated with CD aged 59 years.](image)
though the telomere length may reflect the history of tissue replication, it is also suggested that mechanisms other than cellular turnover may take part in the regulation of telomere length. One of the mechanisms is a pathway mediated by several biochemical factors like oxygen radicals (14). The structure of chromosomal ends consists of single-stranded overhangs with GGG-triplet repeat sequences, which is a major target of reactive oxygen species (17). Therefore, the telomere length of human tissues may also be influenced by the extent of oxidative stress, and the shortening of telomere length might be prevented by antioxidant agents (18). The presence of oxidative stress was found in both HC and DM. Our previous study indicated that plasma hydroperoxide is increased in HC as a result of oxidative stress even when not complicated with CD (19).

It has been reported that inflammatory responses play an important role in the formation of atherosclerotic lesions (20) and may accelerate the telomere attrition of vascular endothelial cells, leading to atherosclerosis (10). These conditions induce premature cell senescence and alteration of cell function such as increased expression of plasminogen activator inhibitor type I (PAI-I), intracellular adhesion molecule I (ICAM-I), and fibronectin, which may contribute to the development of atherosclerosis (13, 21). The idea is supported by the observations by Campisi that some gene products are expressed differentially in senescent endothelial cells and play pathogenic roles in atherosclerosis (22). In the injured artery, inflammatory cells participate in the restoration of endothelium and they also have a high cell turnover. Therefore, telomere shortening of peripheral blood mononuclear cells could be an index of increased cell turnover and the biological age of all cell types related to atherosclerosis.

Another explanation is the participation of genetic factors common to both CD and telomere shortening. Telomere length might be determined genetically, because it is highly heritable (5). In fact, telomere length in peripheral blood mononuclear cells is variable and intrinsically determined even among healthy individuals of the same age (23). If short telomeres are inheritable in CD patients, telomeres might play a primary role in the development of CD by presently unknown mechanisms.

In conclusion, telomere length shortening of peripheral blood mononuclear cells appeared in relation to CD in patients with metabolic diseases such as HC and DM.

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References