Improvement of Endothelial Vasomotor Dysfunction by Treatment With Alpha-Tocopherol in Patients With High Remnant Lipoproteins Levels

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OBJECTIVES
This study sought to examine whether oral intake of alpha-tocopherol, an antioxidant, could improve endothelium-dependent vasorelaxation in patients with high remnant lipoproteins levels.

BACKGROUND
Remnant lipoproteins are known to be atherogenic and impair endothelium-dependent arterial relaxation, but the underlying mechanisms remain unclear. Oxidative stress is a common feature of various risk factors for atherosclerosis.

METHODS
Flow-mediated vasodilation of the brachial artery during reactive hyperemia was examined by high resolution ultrasound technique before and at the end of 4 weeks treatment with oral administration of alpha-tocopherol acetate (300 IU/day) or placebo, which was randomly assigned, in 40 patients with high serum levels of remnants and in 30 patients with low remnants levels in the fasting state (>75th percentile and <25th percentile, respectively, of the distribution of remnants levels in 150 consecutive hospitalized patients).

RESULTS
Before treatment, flow-mediated vasodilation was lower in patients with high remnants levels than in those with low levels (4.1 ± 0.3% vs. 6.0 ± 0.5%, p < 0.01). Treatment with alpha-tocopherol but not with placebo significantly increased flow-mediated dilation in patients with high remnants levels (7.5 ± 0.4% after alpha-tocopherol vs. 4.2 ± 0.4% after placebo, p < 0.01). In patients with low remnants levels, alpha-tocopherol was not effective. The beneficial effect with alpha-tocopherol in high remnants patients was associated with decrease in plasma levels of thiobarbituric acid reactive substances, an indicator of lipid peroxidation (6.6 ± 0.3 nmol/ml before alpha-tocopherol vs. 4.6 ± 0.3 after alpha-tocopherol, p < 0.05).

CONCLUSIONS
Alpha-tocopherol improved impairment of endothelium-dependent vasodilation in patients with high remnants levels. The increase in oxidative stress may at least partly contribute to endothelial vasomotor dysfunction in patients with high remnants levels. (J Am Coll Cardiol 1999;33:1512–8) © 1999 by the American College of Cardiology
tion in patients with high levels of remnant lipoproteins. Flow-mediated vasodilatation in brachial artery has been used as a noninvasive method to assess endothelial function (7,8,11,12).

Thus, this study examined effects of oral administration of alpha-tocopherol, an antioxidant, for four weeks on endothelial vasomotor functions in brachial artery of patients with high and low serum levels of remnants lipoproteins in a randomized and placebo-controlled manner.

METHODS

Study population. The study population included 40 consecutive patients with high serum levels of remnant lipoproteins and 30 consecutive patients with low remnants levels, all of whom underwent diagnostic coronary angiography for atypical chest pain in Kumamoto University Hospital. This study excluded patients treated with lipids-lowering drugs, pharmacologic doses of other antioxidants, angiotensin-converting enzyme inhibitors or calcium channel blocking agents at least a month before and during this study. Clinical features of the study patients are shown in Table 1. Beta-adrenergic blocking agents, nitrates or diuretics were used in some of the study subjects with coronary artery disease or hypertension before and throughout this study, but there was no significant difference in the rates of each of these medications used among the study subgroups. Type III hyperlipoproteinemia was found in two of the patients with high remnants levels. Diabetes mellitus was complicated in 14 of the patients with high remnants levels. The exact cause of high remnants levels was unknown in the remaining patients. Patients with previous myocardial infarction, congestive heart failure, valvular heart disease or other serious diseases were also excluded from this study. High and low serum levels of remnants lipoproteins were determined as levels >75th percentile (>5.1 mg cholesterol/dl) and ≤25th percentile (<2.4 mg/dl), respectively, on the basis of the distribution of serum remnants levels in 150 consecutive patients hospitalized in the Cardiology Section of this hospital, as described in our previous report (4). Written informed consent was obtained from all patients before the study. This study was in agreement with the guidelines approved by the ethics committee at our institution.

Study protocol. The study patients were randomly assigned to have oral intake for 4 weeks of alpha-tocopherol acetate (300 IU/day, 20 and 15 patients with high and low remnants levels, respectively) or placebo (similar-appearing placebo tablet, 20 and 15 patients with high and low remnants levels, respectively) using a random number table generated by a computer. All of the patients were blinded to the content of the tablet. Our preliminary data showed that this dose of alpha-tocopherol increased its content in the isolated remnants by two- or threefold. They were advised to adhere to their usual diet and lifestyle throughout four weeks. All of the study patients completed the trial. Measurements of vasoactivity in the brachial artery and blood sampling were performed in overnight fasting state on the same morning before and at the end of the treatment, in exactly the same manner before and at the end of the treatment. All medications were withdrawn 12 h before the measurements.

Table 1. Clinical Characteristics of Alpha-Tocopherol– and Placebo-Treated Groups in Patients With High and Low Remnants Levels

<table>
<thead>
<tr>
<th></th>
<th>High Remnants (n = 40)</th>
<th>Low Remnants (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alpha-Tocopherol (n = 20)</td>
<td>Placebo (n = 20)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>58.3 ± 2.1</td>
<td>59.6 ± 2.1</td>
</tr>
<tr>
<td>Men/women</td>
<td>8/12</td>
<td>9/11</td>
</tr>
<tr>
<td>Smokers</td>
<td>8 (40.0)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>7 (35.0)</td>
<td>8 (40.0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (30.0)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (35.0)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>3 (15.0)</td>
<td>3 (15.0)</td>
</tr>
<tr>
<td>One-vessel disease</td>
<td>1 (5.0)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Two-vessel disease</td>
<td>2 (10.0)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.3 ± 2.9</td>
<td>23.4 ± 2.8</td>
</tr>
</tbody>
</table>

Values are expressed as mean value ± SE (in age and body mass index) and number of patients. Values in parentheses indicate % of total patients. Smoker, ≥10 cigarettes/day for 10 years; hypercholesterolemia ≥240 mg/dl of serum cholesterol levels; hypertension, blood pressure ≥140/90 mm Hg or on an antihypertensive medication; diabetes mellitus, fasting blood glucose ≥140 mg/dl or on an antidiabetic medication.
Measurements of flow-mediated dilation in brachial artery. Vasodilator responses in the brachial arteries were measured by use of B-mode ultrasound images with a 7.5-MHz linear array transducer (SSH-160A ultrasound system, Toshiba Corp., Tokyo, Japan) as validated previously by our and other studies (7,8,11,12). Measurements were performed by two observers who were blinded to the protocols of the study and the subject grouping. The brachial artery was scanned in the antecubital fossa in a longitudinal fashion. Optimal brachial artery images were obtained between 1 and 5 cm above the antecubital crease. This location was marked, and all subsequent images were obtained at the same location. The exact distance of the measured point of the skin surface from the antecubital crease was recorded in each study subject to ensure that the same point of the brachial artery was measured 4 weeks later. Gain setting was optimized at the beginning of the study and was kept constant throughout the recording period. After baseline measurements of the diameter and flow velocity in the brachial artery, a blood pressure cuff placed around the forearm was inflated with a pressure of 250 to 300 mm Hg for 5 min, and then the cuff was released. Diameter measurements during the reactive hyperemia were taken 45 to 90 s after the cuff deflation. Then, sublingual nitroglycerin (300 μg) was administered, and 3 min later the measurements were repeated. Images were recorded on a super-VHS videocassette recorder (model BR-S601M, Victor Corp., Tokyo, Japan), and brachial arterial diameters were measured from the tape with ultrasonic calipers, as described previously (8,11,12). Responses of the vessel diameters to the reactive hyperemia and nitroglycerin were expressed as a percentage increase in the diameter from the baseline value. The diameter responses were assessed at three points along a 10-mm length of the artery, and the diameter responses were averaged. Blood flow was calculated by multiplying the velocity–time integral of the Doppler flow signal by heart rate and the vessel cross-sectional area. Increase in brachial blood flow was calculated as a maximum flow recorded in the first 15 s after the cuff deflation and was expressed as a percentage increase in the flow from the baseline value.

Measurements of remnant lipoproteins levels. Remnant lipoproteins levels were measured in overnight fasting serum. Remnant lipoproteins were isolated by the application of an immunoaffinity mixed gel containing anti–apolipoprotein (apo)A-1 and anti–apoB-100 monoclonal antibodies (Japan ImmunoResearch Laboratories, Takasaki, Japan) as validated in our previous reports (4,5,13). Cholesterol concentrations in the isolated fraction were measured by the enzymatic method (4,5,13). The fasting remnants levels, measured in this study, were found to be well correlated with the postprandial levels, as shown in our previous study (4).

Plasma levels of alpha-tocopherol and thiobarbituric acid reactive substances (TBARS) and measurement of susceptibility of remnants to oxidative modification. Blood sampling was performed just before the ultrasound study. The plasma levels of alpha-tocopherol were determined by high performance liquid chromatography (14). The plasma level of TBARS, an indicator of lipid peroxides in plasma, was determined as previously described (15,16). Briefly, 2.0 ml of trichloroacetic acid–thiobarbituric acid–HCl reagent was added to 1.0 ml of sample and vortexed. To minimize peroxidation during the assay procedure, butyraldehyde hydroxytoluene was added to the thiobarbituric acid reagent mixture. The results were expressed as malondialdehyde equivalent content (nmol malondialdehyde/ml plasma). The susceptibility of remnants to oxidative modification was determined by measuring Cu²⁺-induced formation of conjugated dienes. The conjugated dienes formation in remnants was assayed by monitoring the change in absorbance at 234 nm in a spectrophotometer as described previously (17).

Statistical analysis. Data are expressed as mean ± SEM. The effects of the treatment on lipids profiles, hemodynamic parameters, brachial arterial parameters and plasma alpha-tocopherol and TBARS levels were compared by two-way analysis of variance with repeated measures followed by post hoc testing with Scheffe test by computer statistical software package SAS, version 6.12 (Cary, North Carolina). Difference between two means among the subgroups were performed by two-tailed paired or unpaired Student t test, as appropriate. Difference in frequencies of risk factors among the subgroups was compared using chi-square test. Statistical significance was defined as p < 0.05.

RESULTS

Clinical characteristics of study patients. Clinical features and lipids profiles before treatment were comparable between alpha-tocopherol–treated patients and placebo-treated patients in either patients with high or low remnants levels (Tables 1 to 3). Levels of total cholesterol and triglycerides were significantly higher in patients with high remnants levels than in those with low remnants levels (total cholesterol, 212 ± 15 mg/dl in high remnants patients vs. 186 ± 11 mg/dl in low remnants patients, p < 0.05; triglycerides, 201 ± 19 mg/dl in high remnants patients vs. 101 ± 16 mg/dl in low remnants patients, p < 0.01). After treatments, levels of remnants cholesterol, total cholesterol, high density lipoprotein cholesterol, and triglycerides were
not significantly changed as compared with those before treatments in each of the subgroups (Tables 2 and 3).

**Flow-mediated dilation in brachial artery.** Before treatment, heart rates, mean blood pressure, baseline arterial diameter of brachial artery, baseline blood flow of brachial artery and percentage increase in the blood flow during reactive hyperemia were comparable between alpha-tocopherol–treated patients and placebo-treated patients in either patients with high or those with low remnants levels (Tables 2 and 3). Flow-mediated dilation of the brachial artery before treatment was significantly lower in patients with high remnant lipoproteins levels as compared with that in those with low remnants levels, but it was comparable between alpha-tocopherol and placebo groups in patients with high remnants levels as well as those with low remnants levels (percentage increase in arterial diameter from baseline, 4.1 ± 0.2% in high remnants patients with alpha-tocopherol vs. 4.2 ± 0.4% in high remnants patients with placebo, p = 0.67; 6.2 ± 0.7%* in low remnants patients with alpha-tocopherol vs. 5.8 ± 0.3%† in low remnants patients with placebo, p = 0.21; *p < 0.01 vs. high remnants patients with alpha-tocopherol, †p < 0.01 vs. high remnants patients with placebo). The dilator response to sublingual nitroglycerin before treatment was comparable among the subgroups (Tables 2 and 3).

After treatment, neither alpha-tocopherol treatment nor placebo treatment affected heart rates, mean blood pressure, baseline arterial diameter of brachial artery, baseline blood flow of brachial artery and percentage increase in the blood flow during reactive hyperemia in both patients with high and those with low remnants levels (Tables 2 and 3). Treatment with alpha-tocopherol significantly increased the flow-mediated dilation as compared with placebo treatment in patients with high remnants lipoproteins levels (Fig. 1).

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**Table 2.** Effects of Treatments in Patients With High Remnants Levels

<table>
<thead>
<tr>
<th></th>
<th>Alpha-Tocopherol (n = 20)</th>
<th>Placebo (n = 20)</th>
<th>Two-Way Analysis of Variance p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After Treatment</td>
<td>Before</td>
</tr>
<tr>
<td>Remnants cholesterol (mg/dl)</td>
<td>6.5 ± 1.2</td>
<td>6.2 ± 1.1</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>214 ± 9.9</td>
<td>213 ± 9.3</td>
<td>210 ± 10</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>49 ± 4</td>
<td>50 ± 5</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>196 ± 14</td>
<td>189 ± 21</td>
<td>205 ± 22</td>
</tr>
<tr>
<td>Alpha-tocopherol (μmol/liter)</td>
<td>23 ± 1.2</td>
<td>51 ± 4.2*</td>
<td>25 ± 1.5</td>
</tr>
<tr>
<td>TBARS (nmol MDA/ml)</td>
<td>6.6 ± 0.3</td>
<td>4.6 ± 0.3†</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>61 ± 5.3</td>
<td>63 ± 4.3</td>
<td>62 ± 5.1</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>88 ± 5.2</td>
<td>90 ± 7.2</td>
<td>88 ± 7.1</td>
</tr>
<tr>
<td>Resting arterial diameter (mm)</td>
<td>3.90 ± 0.19</td>
<td>3.91 ± 0.18</td>
<td>3.88 ± 0.16</td>
</tr>
<tr>
<td>Resting arterial blood flow (ml/min)</td>
<td>188 ± 10</td>
<td>190 ± 9</td>
<td>192 ± 9</td>
</tr>
<tr>
<td>Increase in arterial blood flow (%)</td>
<td>236 ± 15</td>
<td>237 ± 20</td>
<td>234 ± 20</td>
</tr>
<tr>
<td>Dilator response to nitroglycerine (%)</td>
<td>17.3 ± 5.4</td>
<td>17.4 ± 5.7</td>
<td>17.6 ± 5.9</td>
</tr>
</tbody>
</table>

*p < 0.01, †p < 0.05 vs. before alpha-tocopherol using two-way analysis of variance followed by post-hoc testing with Scheffe test. Values are expressed as mean value ± SE. HDL = high density lipoprotein; MDA = malondialdehyde equivalent content; TBARS = thiobarbituric acid reactive substances.”
In patients with low remnants levels, alpha-tocopherol did not significantly increase the flow-mediated dilation as compared with placebo treatment (Fig. 1). Alpha-tocopherol treatment also improved flow-mediated dilation in a subgroup of normocholesterolemic and nondiabetic patients (<240 mg/dl of serum cholesterol levels, <140 mg/dl of fasting blood glucose, no lipids-lowering medication and no antidiabetic medication) with high remnant lipoproteins levels (percentage increase in the arterial diameter, 4.6 ± 0.3% before treatment vs. 7.8 ± 0.8% after treatment, n = 10, p < 0.01). Neither alpha-tocopherol nor placebo treatment changed the dilator response to nitroglycerin in either patients with high or those with low remnants levels (Tables 2 and 3).

Levels of alpha-tocopherol and TBARS and oxidative susceptibility of remnants. Plasma levels of alpha-tocopherol were significantly increased after alpha-tocopherol treatment in both patients with high and those with low remnant lipoproteins levels, whereas the levels were not significantly changed after placebo treatment (Tables 2 and 3). Treatment with alpha-tocopherol but not with placebo significantly decreased plasma levels of TBARS in both patients with high and those with low remnants levels (Tables 2 and 3). The lag time for Cu²⁺-induced conjugated dienes formation of remnant lipoproteins was longer after treatment with alpha-tocopherol as compared with that after placebo (480 ± 58 min after alpha-tocopherol vs. 230 ± 18 min after placebo, n = 5, p < 0.01).

DISCUSSION

The present study demonstrated that flow-mediated vasodilation in brachial arteries was impaired in patients with high remnant lipoproteins levels, a result which is consistent with data observed in human coronary arteries as shown in our previous report (4). The present study further showed that oral administration of alpha-tocopherol for four weeks increased flow-mediated vasodilation in brachial artery of patients with high remnant lipoproteins levels as compared with placebo treatment. The present study also showed that alpha-tocopherol treatment did not affect vasodilation in response to nitroglycerin, an endothelium-independent vasodilator, in either group of patients. Thus, the present study indicates that alpha-tocopherol improved endothelium-dependent arterial dilation in patients with high remnant lipoproteins levels. The improvement of the endothelial dysfunction is unlikely to be due to changes of lifestyle and habits, since profiles of plasma lipids, blood pressure and habits were not altered after treatment as compared with those before treatment, and placebo treatment in the otherwise identical protocol as alpha-tocopherol treatment did not affect the endothelial vasomotor function.

Oxidative stress and remnant lipoproteins. Alpha-tocopherol, a lipid soluble antioxidant, has been shown to protect endothelial cells against oxidative damage (18–21). Previous reports showed that alpha-tocopherol supplementation restored endothelial function in parallel with suppression of lipid peroxidation in low density lipoproteins (LDL) in experimental hypercholesterolemic animals (19,21). In this regard, the present study also showed that the improvement of endothelium-dependent vasodilation was associated with decrease in plasma TBARS levels, an indicator of lipid peroxidation, after alpha-tocopherol treatment in patients with high remnants levels. This strongly suggests that the beneficial effect of alpha-tocopherol, as shown in the present study, was at least partly mediated through prevention of endothelial function from oxidative stress-induced injury in patients with high remnant lipoproteins levels. We have previously shown that oxidatively modified LDL causes endothelial dysfunction and has an important role in atherosclerotic impairment of various endothelial functions (15). It has recently been reported that oxidatively modified remnant lipoproteins caused greater formation of foam cells in in vitro experiments than native remnant lipoproteins (22), suggesting that oxidized remnant lipoproteins may also play an important role in atherosclerosis in hyperlipidemic patients. Our preliminary study showed that oxidized remnant lipoproteins exhibited greater impairment of endothelium-dependent relaxation of isolated rabbit aortas than native remnants (unpublished data). In patients with high remnant lipoproteins levels, the prolonged retention of remnants in the circulation, as a result of the delayed hepatic uptake and/or the increased hepatic secretion of VLDL (23,24), may augment susceptibility of remnants to oxidative modification in the arterial intima, leading to increase in injurious effects of remnants on endothelial function. Among antioxidants, alpha-tocopherol is the major antioxidant contained in lipoproteins (25). Thus, the improve-
ment of endothelial function by alpha-tocopherol in the present study may at least partly occur as a result of protection of remnant lipoproteins against oxidative modification in the arterial intima.

In patients with low levels of remnant lipoproteins, the decrease in TBARS after alpha-tocopherol treatment was not associated with an increase in the endothelium-dependent vasodilation as compared with placebo, though endothelium-dependent dilation in these patients was not impaired before treatment on the basis of our data in healthy adults. The results are in agreement with those in the previous reports (7–10) showing that the beneficial effect of ascorbic acid, another antioxidant, on vasomotor function was exerted in only patients with impaired endothelium-dependent dilation. This presumably may be due to less oxidative damage on endothelial function in patients with low levels of remnant lipoproteins. However, it cannot be completely excluded that alpha-tocopherol exerted the beneficial effects on endothelial functions in high levels of remnant lipoproteins independent of its antioxidant action. In this context, it is interesting that alpha-tocopherol is capable of suppressing activity of protein kinase C, which is suggested to play a role in the impairment of endothelium-dependent vasorelaxation in atherosclerotic arterial walls (26, 27).

Hypercholesterolemia and diabetes mellitus, independent factors of endothelial dysfunction, coexisted with high levels of remnant lipoproteins, as shown in the present and previous studies (4, 28). Thus, there is a possibility that the beneficial effect of alpha-tocopherol on endothelial function in patients with high remnants levels may result from the improvement of endothelial function in patients with hypercholesterolemia and/or diabetes mellitus. However, this possibility cannot explain all of the present data, since beneficial effect of alpha-tocopherol was observed also in normocholesterolemic and nondiabetic patients with high remnants levels, as shown in the present study.

Measurement of remnant lipoproteins levels. Remnant lipoproteins are known to have heterogeneous properties (4, 29). Thus, simple and reliable methods to separate remnant lipoproteins had not been established. The current method with immunoaffinity mixed gel containing anti-aP-(apoA-1) and anti-apoB-100 monoclonal antibodies has been reported to be capable of isolating apoE-rich VLDL particles containing apoB-100 together with chylomicron remnants containing apoB-48, neither of which binds to the immunoaffinity gel (4, 5, 13, 29). This unique anti-apoB-100 monoclonal antibody has been shown to recognize apoB-100 in LDL and most VLDL but not in apoE-enriched VLDL (13). Remnants levels in the postprandial state may be a better predictor (2, 30). However, we have recently shown that fasting levels of remnant lipoproteins are an independent predictor for the presence of endothelial dysfunction in human coronary arteries (4). Thus, the measurement of fasting levels of remnant lipoproteins using the current immunoaffinity gel seems to have a good theoretical basis and could be useful to quantify levels of the atherogenic triglyceride-rich lipoproteins.

Previous studies. The results of the present study are compatible with those of previous studies showing beneficial effects of either vitamin C (7–10), a water soluble antioxidant, or alpha-tocopherol (31) and probucol (32), lipid soluble antioxidants on the endothelial function. However, Gilligan and others (33, 34) showed negative results on the effects of antioxidant vitamins. Different backgrounds, such as the associated risk factors in the study population, may possibly cause the incompatible results among the studies.

Study limitations. Many assays are available for measurement of lipid peroxidation, but no single assay accurately reflects free radical generation. Thiobarbituric acid reactive substances measurement is also susceptible to artifacts caused by variation in sample lipid content and iron contamination of reagents. In the present study, we prevented auto-oxidation of the samples by addition of butyLATED hydroxytoluene to the samples.

Conclusions. The present study showed that alpha-tocopherol improved impairment of endothelium-dependent vasodilation in patients with high remnant lipoprotein levels. Oxidative stress may at least partly play a role in the abnormal endothelial vasomotor function in patients with high remnant lipoprotein levels.

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