Association of Mild Hyperhomocysteinemia with Aortic Calcification in Hypercholesterolemic Patients

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Homocysteine is considered to be an independent risk factor for atherosclerosis. Experimental animal models of hyperhomocysteinemia show aortic calcification, suggesting that this disorder is associated with aortic calcification in humans. A total of 28 patients with hyperlipidemia were enrolled into this study. The degree of aortic calcification at the level of the bifurcation and 1 cm proximal to the bifurcation was assessed by computed tomography of the aorta and the association between calcification of the aorta and the plasma level of homocysteine was then analyzed. The mean plasma homocysteine level in 28 patients was 8.7 μM. They were divided into 2 groups, high homocysteine level group (HHL; homocysteine level > 8.7 μM) and low homocysteine level group (LHL; homocysteine level ≤ 8.7 μM). The degree of aortic calcification at the level of the bifurcation differed significantly between the two groups (19.1% vs. 10.5%; p < 0.01). We found that mild hyperhomocysteinemia was associated with aortic calcification, which suggests that interventions to reduce the plasma level of homocysteine may also reduce the severity of aortic calcification. J Atheroscler Thromb, 2001; 8: 91-94.

Key words: Hypercholesterolemia, Mild homocysteinemia, Atherosclerosis, Aorta CT, Aortic calcification

Introduction

Hyperhomocysteinemia is considered to be a risk factor for thromboembolic disease. Several studies have shown that this biochemical abnormality is associated with atherosclerotic vascular disease (1). However, results of recent prospective studies are contradictory, and raise the question of whether homocysteine itself may cause thromboembolic disease (2). Models of hyperhomocysteinemia--induced atherosclerosis have been developed in rabbits and minipigs by feeding high doses of methionine (3, 4). Aortic lesions in these animals showed thickening of intima, fragmentation of the elastic laminae, the deposition of cholesterol and the presence of calcification. Of those, the presence of aortic calcification is usually considered to indicate an advanced state of atherosclerosis (5). However, the mechanism of such calcification is not fully understood (6, 7). To elucidate the effects of homocysteine on aortic calcification, we measured the extent of calcification in the human aorta using computerized tomography(CT) (8), and determined the plasma level of homocysteine, in addition to evaluating the conventional cardiovascular risk factors. The association between the plasma level of homocysteine and the extent of aortic calcification was analyzed.

Subjects

We evaluated 28 Japanese patients (7 men and 21 women, mean age 61.8 ± 6.7) with hyperlipidemia who...
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gave informed consent to participate in this trial. We excluded subjects with a history of myocardial infarction, cerebrovascular disease, peripheral vascular disease or with uncontrolled diabetes mellitus. A physical examination including the determination of blood pressure and weight was performed for all subjects. Subjects were also asked about their history of smoking.

Methods

Blood was taken from each subject after a 12-hour fast. Serum levels of total cholesterol (TC) and triglyceride (TG), HDL cholesterol (HDL-c) were measured by the autoanalyzer. Serum levels of apolipoproteins A1, B, E and Lp(a) were determined by TIA. Serum levels of intact parathyroid hormone (PTH) were determined by enzyme-linked immunosorbent assay (ELISA). The plasma level of homocysteine was measured by high pressure liquid chromatography (HPLC). The status of coagulation and fibrinolysis was determined. The fibrinogen level was measured by means of the thrombin clotting time, plasminogen was measured by an chromogenic substrate method, the thrombin-antithrombin complex (TAT) and plasminogen activator inhibitor-1 (PAI-1) were measured by enzyme immunoassay (EIA) and the alpha₂ plasmin inhibitor-plasmin complex (PIC) was measured by latex photometric immunoassay (LPIA).

Aortic calcification was evaluated using CT. Briefly, the site of the bifurcation (Bif) and 1 cm proximal to the bifurcation (Prox) of the aorta were scanned by CT with and without contrast media (Omnipaque®). The severity of aortic calcification was determined by the computer-assisted method developed by Tsushima et al. (8). The ratio of the calcified area to the total area of the aorta at the site was calculated and designated as a percent calcified area (%CA). The thickness of the aortic wall was defined as the difference between the total area of aorta and the area filled by contrast media. The ratio of wall thickness to the total area of aorta at the site was calculated and designated as a percent wall thickness (%WT).

The data was reported as mean ± standard deviation (SD). Unpaired t-test was used to compare the two groups. The Mann-Whitney test was used to compare the extent of calcification between the two groups. Fisher’s exact test was used to compare gender and smoking habits between the two groups. A level of P < 0.05 was considered to be significant. The software program, Statview (ver 4.5 ; Hulinks), was used for all analysis.

Results

The general characteristics of subjects are indicated in Table 1. The mean plasma level of homocysteine in subjects was 8.7 ± 3.1 μM. The mean level of total cholesterol was 258.7 ± 22.3 mg/dl (6.68 ± 0.58 mM); that of triglyceride 169.7 ± 159.4 mg/dl (1.49 ± 0.36 mM) and that of HDL-c 57.6 ± 14.0 mg/dl (1.92 ± 0.80 mM). The levels of fibrinogen, plasminogen, creatinine, fasting blood glucose and intact PTH were within normal ranges. The mean body mass index was 22.9 ± 3.0; the mean systolic blood pressure was 135.9 ± 19.4 mmHg; the mean diastolic blood pressure was 78.6 ± 9.7 mmHg. The median %CA at bifurcation was 1.4% and that of %WT was 16.5%. The distribution of %CA and %WT was not normal. The
28 subjects were divided into 2 groups: one group with a high homocysteine level (HHL) that exceeded the mean level of 8.7 μM and the other group with a low homocysteine level (LHL) that was below the mean level of 8.7 μM. The plasma level of homocysteine was significantly higher in the HHL compared to the LHL group (11.0±3.2 μM vs 6.8±1.2 μM; p<0.001). The characteristics of the two groups are shown in Table 2. They did not differ significantly by age, BMI or BP. The lipid parameters, and the indices of coagulation and fibrinolysis also did not differ significantly between the two groups. The serum level of creatinine was significantly higher in the HHL compared to the LHL group (0.81±0.21 mg/dl vs 0.66±0.10 mg/dl; p<0.01). However, the level of intact PTH did not differ between the groups. The mean %CA at bifurcation was 19.1% in HHL and 10.5% in the LHL group, with a significant difference (p<0.01). The mean %CA at Prox also differed significantly between the two groups; 18.3% for HHL vs 11.1% for LHL; p=0.022). However, the mean %WT at bifurcation was 14.4% in HHL and 13.8% in LHL, and that at the Prox was 13.9% in HHL and 14.1% in LHL, with no significant difference between the two groups (p>0.05).

**Discussion**

In this study we found a greater severity of aortic calcification in patients whose plasma levels of homocysteine were over 8.7 μM than that in those with low levels of homocysteine. The definition of hyperhomocysteinemia remains to be established, but Selhub et al. proposed hyperhomocysteinemia as over 14 μM (9). Thus, based on this definition we considered that the level of HHL was mildly high homocysteine in the patients in this study. The thickness of the aortic wall did not differ between the two groups. Other differences found between the two groups involved the serum level of creatinine. Since clearance of homocysteine via the
kidney contributes to its plasma concentration (10), we expected the creatinine level to be higher in patients with hyperhomocysteinemia. Hyperparathyroidism is known to be induced by renal failure, leading to the deposition of calcium in the soft tissue (11). However the level of PTH did not differ between the groups, suggesting that the elevated creatinine level did not contribute to aortic calcification in the patients with HHL.

Atherosclerotic calcification is an organized process that resembles bone formation. Calcium deposits are commonly found in advanced atherosclerotic lesions, and calcification becomes a dominant finding in complicated atherosclerotic lesions (7). A variety of molecules are associated with bone formation, including osteocalcin, osteopontin, osteonectin and bone morphogenetic protein-2a. These molecules have also been shown to be present in atherosclerotic lesions (12, 13). These molecules are synthesized by the smooth muscle cells, foam cells derived from the macrophage, and the pericytes that make up the vascular wall (14-16). The cytokines and growth factors found in the atherosclerotic lesions stimulate the synthesis of such molecules. Risk factors for atherosclerotic calcification include older age, female gender, high level of cholesterol, low level of HDL cholesterol, smoking, obesity, and diabetes (6). Calcification of the artery is seen in experimental hyperhomocysteinemia (3), as well as in patients with homocysteinuria (17). However, since there are few reports of patients with arteries or aortae of with mild hyperhomocysteinemia, it is not known whether mild hyperhomocysteinemia causes the calcification of artery and aorta. The mechanism of the aortic calcification induced by homocysteine remains to be fully elucidated. The fraying of the elastic fibers and the increased deposition of glycosaminoglycan may be associated with such calcification (17). Further study of the effects of homocysteine on the biosynthesis of osteogenesis-related proteins of osteopontin and osteocalcin, are indicated.

In summary, we report that a mildly high plasma level of homocysteine was associated with a high extent of aortic calcification in the patients in this study. This finding suggests that the interventions to reduce the plasma level of homocysteine, such as supplementation of folate, vitamin B6, may be useful in reducing the severity of arterial calcification.

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References