The Role of Cysteine and Homocysteine in Venous and Arterial Thrombotic Disease

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Abstract

Hyperhomocysteinemia is a risk factor for arterial and venous thrombosis, whereas few data are available on the total cysteine (tCy) levels in thrombophilic patients. We studied 82 patients with a previous myocardial infarction (MI; group 1), 68 patients with a previous deep venous thrombosis (group 2), and 100 control subjects (group 3). We assayed total homocysteine (tHcy) and tCy levels by high-performance liquid chromatography with fluorimetric detection. The odds ratios (ORs) for high levels of tCy and tHcy in venous thrombosis and MI were markedly increased in group 1 (fasting tCy: OR, 3.6; 95% confidence interval [CI], 1.6-11.2; postmethionine tCy: OR, 0.97; CI, 0.3-4.0; fasting tHcy: OR, 8.3; CI, 3.9-18.6; postmethionine tHcy: OR, 12.5; CI, 6.8-27.2) and in group 2 (fasting tCy: OR, 2.9; CI, 1.1-7.8; postmethionine tCy: OR, 0.86; CI 0.2-2.6; fasting tHcy: OR, 8.0; CI 3.6-18.0; postmethionine tHcy: OR, 11.0; CI, 6.0-22.1). Our data suggest that plasma tCy levels are a risk factor for venous thrombosis and MI independently of tHcy levels and that it may be appropriate to study both variables simultaneously to thoroughly study the methionine metabolism.

Moderate hyperhomocysteinemia is an established risk factor for arterial and venous thrombosis. The thrombogenicity of total homocysteine (tHcy) resides in its ability to modify the endothelial resistance to thrombosis by several mechanisms. Among these are the induction of an oxidative stress, which enhances the tissue factor expression on endothelial cells; the increase of platelet thromboxane production; and the promotion of the antifibrinolytic effect of lipoprotein (a) plasma levels. In addition, it was demonstrated that short-term exposure of smooth muscle cells to tHcy increases DNA synthesis, induces cyclin A gene expression, and favors the release of mitogenic factors, leading to cell proliferation and migration.

tHcy is derived from methionine metabolism, and it can be degraded through 2 enzymatic pathways: transsulfuration and remethylation. In the remethylation pathway, tHcy receives a methyl group from the folate cycle to be reconverted into methionine. The methionine so formed is activated by adenosine triphosphate to form S-adenosylmethionine, which serves as a methyl donor. In the transsulfuration pathway, tHcy is condensed with serine to form cystathionine by cystathionine beta-synthase, which needs the active form of vitamin B6 as cofactor. Subsequently, cystathionine is converted to total cysteine (tCy) through another vitamin B6-dependent reaction.

The main properties of the amino acid tHcy lie in the presence of a sulfhydryl group in its molecule. Similarly, tCy is a thiol-containing amino acid, which, in vitro studies, mimics many of the chemical properties of tHcy.

Few data are available on the tCy levels in thrombophilic patients. In addition, no data are present in the literature about the behavior of the tCy levels in thrombophilic patients after methionine loading, which is the common test used to
reveal alterations in methionine metabolism. To study these issues, we assayed tCy and tHcy plasma levels, both in the fasting state and after methionine loading, in patients with venous thrombosis or myocardial infarction (MI) in comparison with healthy control subjects.

Materials and Methods

Subjects
We included 3 groups of subjects in the study: group 1 included 82 patients (50 men, 32 women) with a previous acute MI; group 2 included 68 patients (30 men, 38 women) with a previous deep venous thrombosis; and group 3 included 100 healthy control subjects (51 men, 49 women) in the same age bracket, with a "normal" lifestyle and no limitations on their physical activity. Control subjects were recruited from the hospital and university staff, and none had clinical or laboratory evidence of renal, hepatic, or coronary artery disease or a personal or family history of venous thromboembolism.

To rule out possible acute phase–related alterations on the variables studied, we studied all patients at least 6 months after MI or deep venous thrombosis. Patients and control subjects were enrolled in the study after giving informed consent for the use of part of their blood samples for an experimental study.

Experimental Procedures

Venous blood samples for tHcy and tCy determinations were obtained after an overnight fast and 4 hours after methionine loading. We administered 100 mg/kg of body weight of L-methionine in approximately 200 mL of fruit juice immediately after the fasting phlebotomy.

Whole blood was collected in tubes containing an EDTA concentration of 0.17 mol/L, immediately put on ice, and centrifuged within 30 minutes at 4°C (1,500 g for 15 minutes). The supernatant was stored in aliquots at −80°C until assay.

The plasma levels of total tHcy (free and protein bound) and tCy were determined by high-performance liquid chromatography (LKB 2248 pump, Pharmacia, Uppsala, Sweden) and fluorescence detection (Waters 474, Waters Corp, Milford, MA) according to the modification of the Araki and Sako method performed by Legnani et al. The original method of separation was modified by applying ion-pair reversed chromatography.

Briefly, 100 µL of plasma was incubated with 10 µL of 10% tri-n-butylphosphine in dimethylformamide at 4°C for 30 minutes to reduce thiols and release them from plasma proteins. Then, 100 µL of a 0.6 mol/L solution of trichloroacetic acid in a 1-mmol/L concentration of EDTA 2Na+ were added. The mixture was kept for 10 minutes at room temperature and then centrifuged in an Eppendorf microcentrifuge (Eppendorf Scientific, Westbury, NY) at 10,000 rpm for 5 minutes. After a 60-minute incubation in a 60°C water bath, aliquots were cooled to room temperature and analyzed by high-performance liquid chromatography. Separation was carried out at room temperature at a flow rate of 1.2 mL/min.

Standard calibration solutions at different concentrations of tHcy and tCy were obtained by further diluting a stock solution containing homocysteine (1-mmol/L concentration) and cysteine (0.5-mmol/L concentration) prepared in a 0.01-mol/L concentration of hydrochloric acid.

The intraday and interday coefficients of variation were 1.6% and 4.0% respectively.

Statistical Analysis

Unless otherwise indicated, the results are given as median (range). The nonparametric Mann-Whitney test for unpaired data was used for comparisons between single groups. The Spearman rank correlation coefficient was used for the correlation analysis. Multivariate analysis was used to describe the relation of plasma tHcy and tCy concentrations with venous thrombosis or MI. Logistic regression was used with venous thrombosis or MI as the dependent variable and age, sex, and tCy and tHcy concentrations as the independent variables. All odds ratios are given with the 95% confidence interval. All probability values reported were 2-tailed, with values of less than .05 considered statistically significant.

Results

Clinical characteristics of patients and control subjects are given in Table 1.

Cysteine Levels

Fasting tCy levels were significantly higher in patients than in control subjects Table 2. No significant difference was detected between groups 1 and 2. Hypercysteinemia, defined as a tCy concentration above the 95th percentile of the value for control subjects (311 µmol/L) was diagnosed in 17% of patients in group 1 and in 15% of patients in group 2. Similarly, postmethionine tCy levels were significantly higher in patients than in control subjects. No significant difference was detected between groups 1 and 2. After methionine loading, cysteine levels were decreased, even if the difference was not statistically significant. Hypercysteinemia (301 µmol/L) after methionine loading was diagnosed in 14% of patients in group 1 and in 12% of patients in group 2 Figure 1.
The odds ratios adjusted for age, sex, and hyperhomocysteinemia were increased markedly in groups 1 and 2 (Table 3). A positive correlation was found between tCy and tHcy plasma levels ($r = 0.53; P < .001$).

In total, 86 of 150 patients had high levels of tHcy—both fasting and after methionine loading. Twenty-five of 150 patients had high levels of tCy—19 both fasting and postmethionine, 6 only fasting. Nine patients had both hypercysteinemia and hyperhomocysteinemia. Therefore, 16 of 150 patients showed only high tCy levels.

**Discussion**

We analyzed both tCy and tHcy plasma levels before and after a methionine-loading test to accurately study possible derangement of the methionine metabolism in patients with a previous episode of venous thrombosis or MI compared with a group of healthy control subjects.

For tCy levels, we documented a higher prevalence of hypercysteinemia in patients compared with control subjects. In particular, 25 of 150 patients, with a prevalence of 16.7%,
showed tCy plasma levels above the 95th percentile of the distribution for control subjects. As 9 of these patients had also high levels of Hcy, 16 patients had only high levels of tCy with a prevalence of 10.7%. Therefore, by adding the tCy assay to the determination of Hcy, we were able to identify about 11% of patients with an alteration in methionine metabolism, which might be an important, further thrombophilic risk factor. Indeed, high levels of tCy were, at the multivariate analysis adjusted for age, sex, and hyperhomocysteinemia, a risk factor for MI and venous thrombosis independent of Hcy levels.

For tHcy levels, our results confirm data in the literature1-6,23,24 documenting a high prevalence of hyperhomocysteinemia in thrombophilic patients.

Indeed, in total, we diagnosed elevated levels of tHcy in 86 of 150 patients with a prevalence of 57.3%: 13 patients (15%) with high levels of only fasting tHcy; 38 patients (44%) with high levels of fasting and postmethionine tHcy; and 35 patients (41%) with high levels of tHcy only after methionine loading. Therefore, in our group of patients, the performance of methionine loading permitted us to diagnose about 40% of hyperhomocysteinemic patients who would not have been identified without this test.

We did not observe significant differences between groups 1 and 2. This result stresses the relevance of hyperhomocysteinemia as a risk factor for both venous thrombosis and MI and the importance of performing the methionine loading test to evaluate both fasting and postmethionine Hcy levels.

To our knowledge, this is the first report of data about the behavior of tCy after methionine loading in thrombophilic patients. Dose-response experiments in rats showed a decrease of tCy after 2 hours from the administration of 1 or 2 g/kg of methionine.25 Similarly, in healthy men, a rapid decrease in tCy was demonstrated 2 hours after methionine loading and a subsequent increase within 12 hours after loading when tCy approached preloading concentrations.26

In our patients, as in the control group, median tCy levels 4 hours after methionine loading showed a trend toward lower levels in all groups, and, in total, a lower percentage of patients had postmethionine tCy levels above the 95th percentile for control subjects with respect to the fasting tCy levels.

Our data on tCy levels extend the results of previous studies. Indeed, a hypercysteinemia was documented in patients with hyperlipidemia and coronary artery disease, with peripheral vascular disease, and after an MI.18,19 On the other hand, only preliminary data are available on the association of this variable with venous thromboembolism.20

The biologic plausibility of these results is, at the moment, to be ascribed to the properties of this thiol-containing amino acid in in vitro studies. It has been demonstrated that tCy is highly toxic to cultured cells in concentrations about 4-fold higher than normal circulating levels. Similarly to tHcy, it is an easily oxidizable compound, which gives rise to the production of free radicals, thus promoting oxidative damage and, maybe, enhancing the expression of tissue factor on endothelial cells.16,27

A limitation of the present study was the lack of information about the vitamin status of patients and control subjects. However, other authors have documented no relationship between cysteinemia and vitamin B12 or folate.28 In addition, the study design (case-control study) can reveal associations, not causality, among the variables studied.

**Table 3**

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<tr>
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<th>Fasting Hcy</th>
<th>Postmethionine Hcy</th>
<th>Fasting Cy</th>
<th>Postmethionine Cy</th>
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<tr>
<td>Group 1</td>
<td>8.3 (3.9-18.6)†</td>
<td>12.5 (6.8-27.2)‡</td>
<td>3.6 (1.6-11.2)§</td>
<td>0.97 (0.3-4.0)‡</td>
</tr>
<tr>
<td>Group 2</td>
<td>8.0 (3.6-18.0)†</td>
<td>11.0 (6.0-22.1)‡</td>
<td>2.9 (1.1-7.8)§</td>
<td>0.86 (0.2-2.6)‡</td>
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Cy, cysteine; Hcy, homocysteine.
† Data are given as odds ratio (95% confidence interval). The groups are as follows: 1, myocardial infarction; 2, deep venous thrombosis.
‡ P < .0001.
§ P < .05.
P not significant.
Our results suggest that plasma tCy levels are a risk factor for venous thrombosis and MI independently of tHcy levels and that it may be appropriate to simultaneously study tCy and tHcy levels to thoroughly study homocysteine and methionine metabolism. Further studies are required to evaluate the association of tCy levels with the other known risk factors for thrombophilia. Prospective studies seem warranted to further investigate the relationship between cysteine and thrombosis.

References