ORIGINAL ARTICLE

Korean Circulation J 2004;34(1):53-58

Elevated Serum Homocysteine in Patients with Coronary Vasospasm

Seong-Woo Han, MD, Kyu-Hyung Ryu, MD and Yung Lee, MD

Division of Cardiology, Department of Medicine, Hallym University, Anyang, Korea

ABSTRACT

Background: Homocysteine is a metabolite of methionine with atherogenic properties via endothelial dysfunction. Endothelial dysfunction is one of main pathophysiologic mechanisms of coronary vasospasm. We examined the relationship between the risk of patients with coronary vasospasm and serum total homocysteine (tHcy), folate, vitamin B_{12}, and plasma vitamin B_6. **Methods**: Fasting serum tHcy, folate, vitamin B_{12}, and plasma vitamin B_6 concentrations were measured in 25 patients (15 men, age 52.8±10.1) with coronary vasospasm and compared with 22 healthy control subjects matching in age and sex (10 men, age 52.7±9.2). Serum tHcy concentration was higher in the vasospasm group than in the control group (14.8±5.3 vs 10.1±2.5 μmol/L, $p<0.001$). **Results**: Serum folate (6.3±1.0 vs 10.2±4.5 ng/mL, $p<0.001$) and vitamin B_{12} concentration (544.8±181.7 vs 1004.9±567.1 pg/mL, $p<0.001$) were lower in vasospasm group. There was no significant difference in plasma vitamin B_6 concentration between the two groups (77.8±44.3 and 95.8±63.4 nmol/L). **Conclusions**: These data support the hypothesis that elevated serum tHcy is a risk factor for coronary vasospasm. Low folate and vitamin B_{12} levels in patients with coronary vasospasm suggest that these agents contribute therapeutically to the treatment of patients with coronary vasospasm. **(Korean Circulation J 2004;34(1):53-58)**

KEY WORDS: Homocysteine; Coronary disease; Coronary vasospasm.

Introduction

Homocysteine is a sulfur containing amino acid derived from the metabolism of methionine and has athergogenic and thrombogenic properties. It is regarded as a risk factor for artherosclerotic coronary artery disease.[1-4] Homocysteine is readily oxidized, leading to the formation of superoxide and hydrogen peroxide. This oxidant stress may play an important role in endothelial damage, which occurs easily before the development of overt structural changes in coronary artery disease.[5][6] Several animal and clinical studies have shown homocysteine to reduce the bioavailabilty of endothelium-derived relaxing factors (EDRF) or endothelium derived nitric oxide (NO).[7] Increased blood homocysteine levels are associated with impaired flow-mediated vasodilation at the brachial artery.[8-10] Flow-mediated vasodialtion of the brachial artery is a good indicator of endothelial function and was reported to correlate with coronary circulation.[11]

Vasospasm of the coronary artery can cause various features of ischemic heart disease syndrome, including variant angina, myocardial infarction, and even sudden cardiac death.[12] The deficiency of endothelial nitric oxide (NO) activity plays an important role in the pathogenesis of coronary vasospasm.[13] Therefore, we hypothesized that mild to moderate elevations in the serum total homocysteine concentrations (tHcy) are associated with decreased nitric oxide bioavailability and coronary vasospasm. Accordingly, we compared the level of fasting serum tHcy and related substrates to the level of homocysteine in patients with coronary vasospasm and in control subjects.

Received : May 30, 2003
Accepted : December 31, 2003
Correspondence : Kyu-Hyung Ryu, MD. Departmnet of Medicine, Hallym University Hospital, Pyungchon-dong, Dongan-gu, Anyang 431-070, Korea
Tel : 82-31-380-3722, Fax : 82-31-386-2269
E-mail : khryu@hallym.or.kr

Methods

Study patients

This study included 25 patients who were diagnosed with vasospastic angina between January 1996 and December 1997 at Hallym University hospital. If typical angina symptoms, focal reversible coronary vasospasms, and transient ECG changes of myocardial ischemia occurred during coronary angiogram with ergonovine or during the acetylcholine provocation test, a diagnosis of vasospastic angina was made. All angina patients, due to coronary vasospasms, had angiographically documented reversible coronary vasospasms associated with ischemic ST segment changes, with chest pain or chest discomfort, and had no visible fixed significant stenosis on coronary angiogram. With the 22 control subjects, detailed medical histories were taken and physical examinations were performed. The patients and the control subjects were matched by age and sex. The exclusion criteria contained the following: impaired renal function (serum creatinine over 2.0 mg/dL), anemia, hepatic disease, current use of vitamin compositions, malignancy, and herb medication.

Measurement

Serum and plasma were obtained after overnight fasting and were promptly spun, separated, and frozen at −20℃. High-performance liquid chromatography with fluorescent detection methods was used for the rapid determination of tHcy concentration.[14] Serum folate and vitamin B_{12} levels were measured using radioassay kits. Plasma vitamin B_6 levels were measured by radioenzyme assay. Other laboratory tests included measurements of fasting serum total cholesterol, HDL-cholesterol, triglyceride, lipoprotein (a), and fibrinogen. LDL-cholesterol levels were calculated by the Friedewald equation. Body mass index was calculated by dividing body weight (Kg) by the square of height (m).

Statistical analysis

Continuous variables were expressed as mean±standard deviation, and the Mann-Whitney U test was used to compare means between the two groups. Fisher's exact test was used to compare categorical variables between the groups. Variables that showed significance were included in multivariated logistic regression for evaluating intervariable correlations. P values less than 0.05 were considered statistically significant.

Results

Basic clinical characteristics

The clinical characteristics of the study populations are shown in Table 1. There was no significant difference in age, sex, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, and BMI between the two groups. The frequency of hypertension and diabetes were also similar between the two groups. The history of cigarette smoking, which was higher in the vasospasm group, was the only difference ($p<0.05$).

Serum total homocysteine, folate, vitamin B_{12}, vitamin B_6 concentrations

Serum concentrations of tHcy were higher in patients with coronary vasospasm than in the control subjects (14.8±5.3 versus 10.1±2.5 μmol/L, $p<0.001$, Figure 1). Compared with the control group, the vasospasm group showed lower serum folate concentrations (10.2±4.5

Table 1. Basic clinical characteristics and cardiovascular risk factors

	Coronary spasm (n=25)	Control (n=22)
Age	52.7± 9.2	52.8±10.8
Sex (male)	15	11
Hypertension	6	4
Diabetes	5	6
Smoker*	13	5
BMI (Kg/m^2)	23.6± 3.4	24.1± 3.4
Total cholesterol (mg/dL)	182.1±31.0	199.0±73.0
Triglyceride (mg/dL)	164.0±83.9	154.8±88.9
HDL-cholesterol (mg/dL)	38.6± 8.5	42.9±10.5
LDL-cholesterol (mg/dL)	115.2±25.6	124.0±10.5

*: $p<0.05$ by Fisher's exact test. BMI: body mass index, HDL: high density lipoprotein, LDL: low density lipoprotein

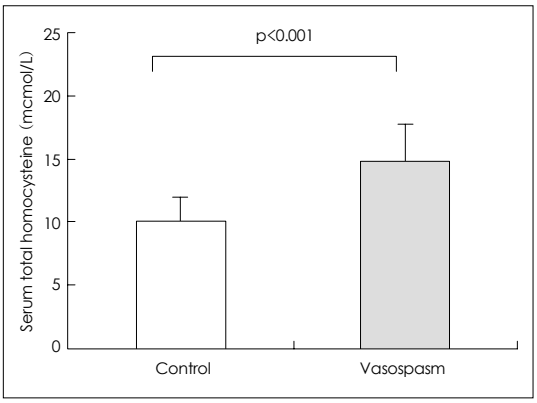

Figure 1. Serum total homocyteine concentrations (tHcy) in control and vasospasm group. Serum tHcy concentration was higher in vasospasm group (14.8±5.3 μmol/L) than that of control subjects (10.1±2.5 μmol/L, p<0.001 by Mann-Whitney U test).

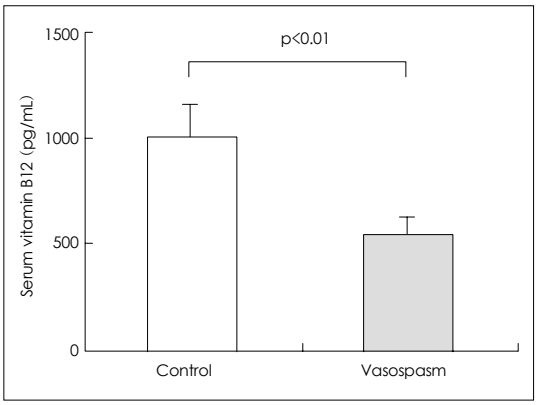

Figure 3. Serum vitamin B_{12} levels in control and vasospasm group. Serum vitamin B_{12} level was lowe in vasospasm group (544.8±80.7 pg/mL) than that of control subjects (1004.9±101.2 pg/mL, p<0.01 by Mann-Whitney U test).

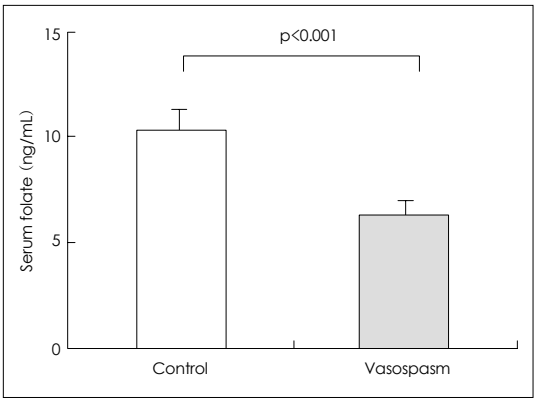

Figure 2. Serum folate levels in control and vasospasm group. Serum folate level was lowe in vasospasm group (6.3±0.7 ng/mL) than that of control subjects (10.2±0.7 ng/mL, p<0.001 by Mann-Whitney U test).

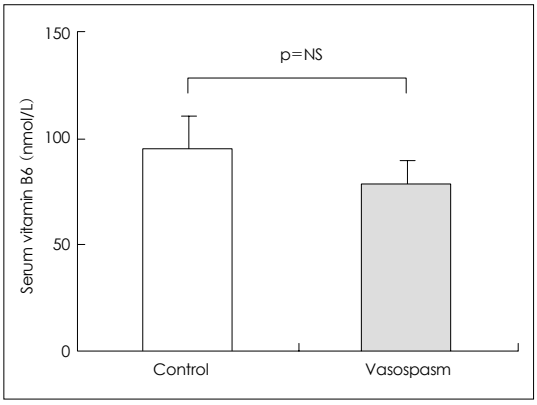

Figure 4. Serum Vitamin B_6 levels in control and vasospasm group. There was no significant difference between two groups. In control group: 95.8±11.8 nmol/L, in vasospasm group: 77.8±11.3 nmol/L.

versus 6.3±1.0 ng/mL, p<0.001, Figure 2) and lower vitamin B_{12} levels (1004.9±567.1 versus 544.8±181.7 pg/mL, p<0.001, Figure 3). No significant difference was observed in plasma vitamin B_6 concentration between the two groups (95.8±63.4 and 77.8±44.3 nmol/L respectively, Figure 4).

Serum total homocysteine concentration and conventional risk factors for cardiovascular disease (Table 2)

The results showed that sex (13.8±5.6 μmol/L and 11.1±3.2 μmol/L in males and females, respectively), diabetes (11.6±3.7 μmol/L and 12.9±5.1 μmol/L in diabetes and non-diabetes patients, respectively) and hypertensiveness (11.4±3.2 μmol/L and 12.5±3.9 μmol/L in hypertensive and non-hypertensive patients, respectively) did not affect the concentration of serum tHcy. The serum tHcy level of smokers and non-smokers also showed no significant difference (13.5±5.1 μmol/L and 12.1±4.6 μmol/L respectively). By logistic regression analysis, cigarette smoking (odd ratio 4.777, 95% confidence interval 1.076−21.202, p<0.05) and serum tHcy concentration (odd ratio 1.302, 95%

Table 2. Serum total homocysteine concentration according to conventional cardiovascular risk factors

Risk factors	Serum total homocysteine (μmol/L)
Sex	
Male : female	13.8±5.6 : 11.1±3.2
Presence of diabetes	
Diabetes : non-diabetes	11.6±3.7 : 12.9±5.1
Presence of hypertensive	
Hypertensive : non-hypertensive	11.4±3.2 : 12.5±3.9
Smoking	
Smoker : Non-smoker	13.5±5.1 : 12.1±4.6

confidence interval 1.064−1.594, $p<0.01$)

Discussion

In the present cross sectional and case-control study, patients with coronary vasospasm showed higher serum total homocysteine concentration than the control patients. This suggests that increased serum homocysteine is associated with coronary vasospasm.

Coronary vasospasm can cause variable clinical features of myocardial ischemia. Despite its clinical significance, the factors responsible for coronary artery hypersensitivity to vasoconstricting stimuli are still unknown. From the results of several studies, cigarette smoking is the most accepted characteristic clinical risk factor for coronary vasospasm, but its association with other conventional risk factors is weak.[15)16)] Similarly, only cigarette smoking showed a significant difference between the two groups in this study. Serum total homocyteine is also independently associated with coronary vasospasm.

Among the mechanisms of pathogenesis of coronary vasospasm, impaired endothelial function is regarded as one of the main pathophysiologic mechanisms. Endothelial dysfunction manifests an impaired bioavailability of endothelium derived relaxing factor (EDRF). EDRF is a diffusible vasodilator from vascular endothelium and causes vascular smooth muscle relaxation. Moreover, reports have suggested nitric oxide (NO) to be the substance of EDRF.[17)] EDRF can be released by platelet products, thrombin, hormones, neurotransmitters, local autacoids, and by change of local blood flow.[18)] The response to variations in shear stress significantly affects the change in diameter of large arteries in response to increased blood flow, which is called flow-mediated vasodilation.[19)] In canine coronary arteries, acute endothelial injury by a balloon-tipped catheter causes enhanced contraction in response to serotonin.[20)] Furthermore, the combination of balloon removal of endothelium with hypercholesterolemic diet caused atherosclerotic lesions and selective vasospasms where the endothelium was removed with myocardial ischemia in the porcine model.[21)] Some data suggest that coronary vasospasm is associated with endothelial dysfunction in human. Reports have also suggested genetic enzyme defects in endothelial NO synthesis to be a risk factor for coronary vasospasm.[22)] Moreover, the endothelium dependent flow-mediated dilation in the brachial artery was impaired in patients with vasospastic angina.[23-25)]

Mild to moderate elevation of serum homocysteine is now regarded as a risk factor for thrombotic and atherosclerotic vascular disease. Homocysteine readily oxidizes, leading to the formation of superoxide and hydrogen peroxide, and this oxidant stress may play an important role in endothelial dysfunction, which is a major pathophysiologic mechanism for artherosclerosis and coronary vasospasm. There have been several reports indicating that hyperhomocysteinemia is related with vascular endothelial dysfunction, which easily occurs before the development of overt structural vascular disease. Harker et al. reported that adding homocysteine to a culture medium caused endothelial injury. Furthermore, they reported that intravenous infusion of homocysteine in baboons caused patch endothelial detatchment.[5)]

No data has shown the association of homocysteine with coronary vasospasm. The above studies, however, demonstrated that increased homocysteine concentration is associated with impaired endothelium-dependent vasodilation, employed at the brachial or femoral arteries to estimate flow mediated endothelium-dependent vasodilation. And the flow-mediated endothelium-dependent

vasodilation in the brachial artery correlated well with the invasively-tested endothelial function of the coronary artery.[26] Therefore, we assumed increased serum homocysteine to induce endothelial dysfunction, causing coronary vasospasm. The results of this study support this assumption .

Homocystinuria is a rare inherited disorder caused by genetically impaired enzyme cystathionine-beta-synthase in homozygote and shows markedly increased homocysteine concentration.[27] But decreased activity of methylenetetrahydofolate reductase (MTHFR) occurs quite frequently, and showed mild to moderately increased homocysteine levels and variable clinical features, including vascular disease.[28] Vitamin B_{12}, vitamin B_6 and folate are essential nutritional cofactors in the metabolism of homocysteine.[29][30] Low levels of these vitamins may result in mild to moderate hyperhomocysteinemia; administration of these vitamins reduces homocysteine levels. In this study, serum folate and vitamin B_{12} levels were lower in the vasospasm group than in the control group. Although the activity of MTHFR was not evaluated, the results of this study suggest that relatively low levels of these vitamins are associated with increased homocyteine levels in patients with coronary vasospasm.

In conclusion, we demonstrated that serum total homocyteine concentration increased in patients with coronary vasospasm. Further studies are needed to explain the causal relationship by which another therapeutic modality for coronary vasospasm can be expected.

■ **Acknoewledgments**

Main result of this study was presented at annual scientific meeting of American College of Cardiology, 1998.

REFERENCES

1) Israelsson B, Brattstorm LE, Hultberg BL. *Homocysteine and myocardial infarction. Atherosclerosis 1988;71:227-33.*
2) Stampfer MJ, Malinow MR, Willette WC, Newcomer LM, Upson B, Ullman D, et al. *A prospective study of plasma homocysteine and risk of myocardial infarction in US physician. JAMA 1992;268:877-81.*
3) Pancharuniti N, Lewis CA, Sauberlich HE, Pekins LL, Go RC, Alvarez JO, et al. *Plasma homocysteine, folate and vitamin B_{12} concentrations and risk factor for early onset coronary artery disease. Am J Clin Nutr 1994;59:940-8.*
4) Nygard O, Nordrehaug JE, Refsum H, Uerland PM, Farstad M, Vollset SE. *Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997; 337:230-6.*
5) Harker LA, Ross R, Slichter SJ, Scott CR. *Homocyteine-induced arteriosclerosis: the role for endothelial cell injury and platelet responses in its genesis. J Clin Invest 1976;58: 731-41.*
6) Wall RT, Harlan JM, Harker LA, Striker GE. *Homocyteine induced endothelial cell injury in vitro: a model for the study of vascular injury. Thromb Res 1980;18:113-21.*
7) Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, et al. *Adverse vascular effects of homocyteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest 1993;91:308-18.*
8) Woo KS, Chook P, Lolin YI, Cheung AS, Chan LT, Sun YY, et al. *Hyperhomocysteinemia is a risk factor for arterial endothelial dysfunction in humans. Circulation 1997;96: 2542-4.*
9) Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. *Hyperhomocysteinemia is associated with impaired endothelium dependent vasodilation in humans. Circulation 1997; 95:1119-21.*
10) Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Bones C, Newcombe RG, et al. *Hyperhomocysteinemia after an oral methionine load acutely impaired endothelial function in healthy adults. Circulation 1998;98:1848-52.*
11) Sorensen KE, Celemajer DS, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Thomas O, et al. *Non-invasive measurement of human endothelium dependent arterial response: accuracy and reproducibility. Br Heart J 1995; 74:247-53.*
12) Maseri A, Severi S, de Nes M, L'Abbate A, Chiercia S, Marzilli M, et al. *"Variant" angina: one aspect of a continuous spectrum of vasospastic myocardial ischemia: pathogenetic mechanisms, estimated incidence and clinical and coronary arteriographic findings in 138 patients. Am J Cardiol 1978;42:1019-35.*
13) Vanhoutte PM, Shimokawa H. *Endothelium-derived relaxing factor and coronary vasospasm. Circulation 1989;80: 1-9.*
14) Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. *Total homocysteine in plasma or serum: methods and clinical applications. Clin Chem 1993;39: 1764-79.*
15) Sugiishi M, Takatsu F. *Cigarette smoking is a major risk factor for coronary spasm. Circulation 1993;87:76-9.*
16) Nobuyoshi M, Abe M, Nosaka H, Kimura T, Yokoi H, Hamasaki N, et al. *Statistical analysis of clinical risk factors for coronary artery spasm: identification of the most important determinent. Am Heart J 1992;124:32-8.*
17) Furchgott RF, Zawadzki JV. *The obligatory role of endothelial cells in the relaxation of arterial smooth muscles by acetylcholine. Nature 1980;288:373-6.*
18) Furchgott RF. *Role of endothelium in responses of vascular smooth muscle. Circ Res 1983;53:557-73.*
19) Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, et al. *Nitric oxide is responsible for flow-de-*

pendent dilation of human peripheral conduit arteries in vivo. Circulation 1995;91:1314-9.
20) Brum JM, Sufan Q, Lane G, Bove AA. *Increased vasoconstrictor activity of proximal coronary arteries with endothelial damage in intact dog. Circulation 1984;70:1066-73.*
21) Shimokawa H, Tomoike H, Nabeyama S, Yamamoto H, Ishii Y, Tanaka K, et al. *Coronary artery spasm induced in miniature swine: angiographic evidence and relation to coronary atherosclerosis. Am Heart J 1985;110:300-10.*
22) Yoshimura M, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Nakamura S, et al. *A T-786 → C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene and coronary arterial vasomotility. Am J Cardiol 2000;85:710-4.*
23) Taragawa H, Kato M, Kurokawa J, Yamagata T, Matsuura H, Chayama K. *Endothelial dysfunction is an independent factor responsible for vasospastic angina. Clin Sci 2001;101:707-13.*
24) Hamabe A, Takase B, Uehata A, Kurita A, Ohsuzu F, Tamai S. *Impaired endothelium-dependent vasodilation in the brachial artery in variant angina pectoris and the effect of intravenous administration of vitamin C. Am J Cardiol 2001;87:1154-9.*
25) Moriyama Y, Tsunodar R, Harada M, Miyao Y, Yoshimura M, Kugiyama K, et al. *Nitric oxide-mediated vasodilation is decreased in forearm resistance vessels in patients with coronary spastic angina. Jpn Circ J 2001;65:81-6.*
26) Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, et al. *Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-41.*
27) Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, et al. *The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet 1985;37:1-31.*
28) Kang SS, Passen EL, Ruggie N, Wong PW, Sora H. *Thermolabile defect of methylenetetrahydrofolate reductase in coronary artery disease. Circulation 1993;88:1463-9.*
29) Stabler SP, Marcell PD, Podell ER, Allen RH, Savage DG, Lindenbaum J. *Elevation of total homocysteine in the serum of patients with cobalamine or folate deficiency detected by capillary gas chromatography mass spectrometry. J Clin Invest 1988;81:466-74.*
30) Verhoef P, Stampfer MJ, Burning JE, Gaziano JM, Allen RH, Stabler SP, et al. *Homocysteine metabolism and risk of myocardial infarction: relation with vitamin B_6, B_{12} and folate. Am J Epidemiol 1996;143:845-59.*