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Different impact of hyperhomocysteinemia on cerebral small vessel ischemia and cervico-cerebral atherosclerosis in non-stroke individuals

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ABSTRACT

Background: Our aim was to investigate the impact of plasma total homocysteine (tHcyt) levels on cervico-cerebral atherosclerosis and cerebral small vessel ischemia in non-stroke individuals.
Methods: Demographic, laboratory, brain magnetic resonance imaging and magnetic resonance angiographic data were retrospectively analyzed in 682 non-stroke individuals. The association between plasma tHcyt and radiological indices of cervico-cerebral atherosclerosis (any presence of cervico-cerebral [aCC] atherosclerosis, extracranial [EC] atherosclerosis and intracranial [IC] atherosclerosis) and cerebral small vessel ischemia (silent brain infarct [SBI] and cerebral white matter hyperintensity [cWMH]) was analyzed after adjusting for cardiovascular risk factors.
Results: There was no association between values for natural log-transformed tHcyt (log-Hcyt) and aCC atherosclerosis, EC atherosclerosis, or IC atherosclerosis. The log-Hcyt was independently associated with cWMH (OR: 3.07, 95% CI: 1.64-5.75) and SBI (OR: 2.91, 95% CI: 1.57-5.40) in multivariate analysis. Median plasma tHcyt level increased as the severity of cWMH increased.
Conclusions: Our results suggest that hyperhomocysteinemia plays a major role in the development of cerebral small vessel ischemia, but not in the development of atherosclerosis of major cerebral arteries.

Introduction

Atherosclerosis of major cranial arteries (cervico-cerebral atherosclerosis) is the most common cause of ischemic stroke. Cervico-cerebral atherosclerosis can be divided into 2 types: extracranial (EC)- and intracranial (IC) atherosclerosis. There is convincing evidence that EC- and IC atherosclerosis have different pathogeneses and are exhibited in patients of different ethnic backgrounds [1]. IC atherosclerosis is common in Asians and Africans, whereas EC atherosclerosis is common in Caucasians. Several studies have failed to find any difference in classic cardiovascular risk factors between EC- and IC atherosclerosis [2,3]. These findings indicate that novel risk factors may be responsible for the different locational distribution of cervico-cerebral atherosclerosis. In addition to traditional cardiovascular risk factors, inflammatory markers such as C-reactive protein [4,5] are associated with carotid stenosis, suggesting that inflammation plays a role in the pathogenesis of atherosclerosis.

Cerebral small vessel ischemia, including silent brain infarction (SBI) and cerebral white matter hyperintensity (cWMH), is occasionally found in healthy people. Emerging evidence demonstrates that it is associated with an increased risk of future stroke [6] and dementia [7]. Distinct from cervico-cerebral atherosclerosis, it has a different pathogenesis, including lipohyalinosis, microatheroma, and segmental demyelination of microvessels in the brain [8]. Recent studies suggest that endothelial dysfunction plays a role in the development of lacunar infarction and cWMH [9,10].

Hyperhomocysteinemia is recognized as a risk factor for major vascular events. One meta-analysis found that a 25% elevation (about 3 μmol/L) in plasma total homocysteine (tHcyt) was associated with about a 10% higher risk of cardiovascular events and a 20% higher risk of stroke [11]. Another meta-analysis found that elevated levels of plasma tHcyt increase the risk of venous thromboembolism [12]. The proposed mechanisms by which hyperhomocysteinemia causes these vascular complications are: the induction of atherosclerosis by homocysteine's interaction with lipids [13]; the induction of vascular endothelial dysfunction by reducing the bioavailability of endothelial nitric oxide synthetase (eNOS) [14]; and the promotion of oxidative stress [15].

Previous clinical observations have indicated that increased plasma tHcyt is associated with indices of subclinical brain injury, including

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SBI [16,17] and cWMH [17,18]. Homocysteine is recognized as an important endothelial toxin in cerebral small vessel ischemia. One experimental study showed that the association between plasma tHcyt levels and cerebral small vessel ischemia was no longer significant after controlling for endothelial markers such as thrombomodulin and intracellular adhesion molecule-1, indicating that elevated tHcyt levels have their effect on cerebral small vessel ischemia by causing endothelial dysfunction [19].

The association between hyperhomocysteinemia and EC atherosclerosis is controversial. Several studies found an association [20,21] but others did not [22]. Previous experimental studies demonstrated that hyperhomocysteinemia does not induce lipid-rich atheroma per se, but it does worsen the atherosclerotic process in the presence of hyperlipidemia [13]. The recent VITAmins To Prevent Stroke (VITATOPS) trial showed that homocysteine-lowering treatment does not reduce the overall risk for stroke recurrence [23]. These findings raise questions about the independent effect of hyperhomocysteinemia on the pathogenesis of atherosclerosis. We previously reported a lack of association between plasma tHcyt and EC- or IC atherosclerosis in stroke patients [24]. However, the true causative effect of hyperhomocysteinemia in ischemic stroke is difficult to determine since plasma tHcyt is known to be elevated in the acute phase of stroke [25]. Besides, there have been no studies investigating the effect of hyperhomocysteinemia on small vessel ischemia and cervico-cerebral atherosclerosis in single population. In the present study, we investigated the different effects of hyperhomocysteinemia on cervico-cerebral atherosclerosis and cerebral small vessel ischemia in a non-stroke population.

Patients and Methods

Study Population

The Institutional Ethical Committee of the CHA Bundang Medical Center approved this study (IRB approval no. 2010–083). The study was designed as a retrospective analysis of 1059 non-stroke individuals, aged ≥ 40 years, who visited the outpatient clinic of the Department of Neurology at CHA Bundang Medical Center for routine health examination between March 2008 and Dec 2010. Subjects presented for medical attention because they had underlying cardiovascular risk factors, or a family history of stroke. We reviewed the medical records, laboratory results, and radiological findings in all subjects. We included only patients whose records contained adequate information concerning demographic, laboratory, and radiological data. Of 1059 patients, we excluded 377 for the following reasons: (1) inadequate medical information (n = 39); (2) no laboratory tests for vascular risk factors (n = 52); (3) No brain magnetic resonance image (MRI) or magnetic resonance angiography (MRA) data (n = 143); (4) previous history of neurological disease including cerebrovascular accident (n = 35); (5) abnormal neurological findings at the time of examination (n = 18); (6) vitamin supplementation (n = 81); (7) non-Korean ethnicity (n = 9). A total of 682 subjects were included in the MRI and MRA analysis.

Risk Factors Assessment

We reviewed patients' medical records in order to gather information on their previous history and laboratory data related to cardiovascular risk factors. Hypertension was defined as a high baseline blood pressure (systolic ≥ 140 mm Hg or diastolic ≥ 90 mm Hg) or a history of antihypertensive medication. Diabetes mellitus (DM) was defined as a fasting plasma glucose of ≥ 126 mg/dL or a history of insulin or oral hypoglycemic therapy. Smoking was defined as current smoking status at the time of examination. Hyperlipidemia was defined as a fasting serum total cholesterol of ≥ 220 mg/dL or a history of treatment with a statin. The category of ischemic heart disease (IHD) included a history of myocardial infarction, unstable angina, coronary angioplasty, or coronary bypass graft surgery.

We collected plasma tHcyt data for all study subjects, including in the study only those tests performed within 1 month of radiological examinations. All blood samples were processed using a standard protocol. Fasting venous blood samples in tubes containing trisodium ethylenediaminetetraacetic acid were promptly centrifuged, and stored at −20 °C. Plasma tHcyt levels were determined using fluorescence polarization immunoassay with IMx (Abbott laboratories, Abbott Park, Ill., USA). The inter-assay coefficient variation was in the range of 3 ~ 6 %.

Assessment of Cervico-Cerebral Atherosclerosis

We reviewed the results of brain MRAs conducted using a 1.5 tesla-MRI system (MAGNETOM Symphony, Siemens, Heidelberg, Germany) in all study subjects. Two neurologists who were unaware of patients' clinical information retrospectively analyzed the MRA data. A diagnosis of EC- or IC atherosclerosis was made only when both investigators concurred.

EC atherosclerosis was defined as stenosis ≥ 50% or total occlusion at the EC portion of the internal carotid artery (ICA) or vertebral artery (VA). Degrees of carotid stenosis were measured using the methods used in the North American Symptomatic Carotid Endarterectomy Trial (NASCET) [26], which were based on the use of time-of-flight images of the carotid arteries. IC atherosclerosis was defined as stenosis ≥ 50% or occlusion at the proximal portion of the middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA), basilar artery (BA), or the intracranial portion of the carotid artery and VA [24]. Assessment of the distal branches of major intracranial arteries was not performed, due to the small caliber of these arteries and a lack of consensus among measurement methods on MRA [27].

Assessment of Silent Brain Infarction and Cerebral White Matter Hyperintensity

Brain MR images of all study subjects were retrospectively examined for SBI and cWMH lesions. Sixteen axial images were obtained using a slice thickness of 7 mm with a 2 mm inter-slice gap. The MR imaging protocol consisted of a T1-weighted image (TR/TE = 560/14 ms), and fluid attenuated inversion recovery (FLAIR) images (TR/TE = 9000/105 ms; inversion time, 2500 ms). Two neurologists, unaware of the patients' clinical information, evaluated each MRI, and SBI was diagnosed only when both investigators agreed. SBI was defined as a small (3–20 mm diameter), cavitated lesion. It appeared hypointense on T1-weighted imaging, and on FLAIR imaging appeared hypointense with a hyperintense rim [28].

The presence of cWMH was evaluated on FLAIR images by the same neurologists. cWMH severity was assessed using the scoring system of Fazekas et al. [29] and divided into 4 grades: grade 0 = absent; 1 = punctuate; 2 = early confluent; and 3 = confluent lesions in the bilateral periventricular and subcortical white matters.

Statistical Analysis

We used the χ^2 test for categorical data and the two-sample *t* test for continuous data, or non-parametric testing (Mann–Whitney U test) when variables did not show a normal standard distribution. We performed logistic regression analysis according to the individual radiological indices of cervico-cerebral atherosclerosis (any presence of cervico-cerebral [aCC] atherosclerosis, EC atherosclerosis, and IC atherosclerosis) and cerebral small vessel ischemia (cWMH and SBI). We conducted univariate- and multivariate logistic regression analysis to evaluate the plasma tHcyt and individual radiological indices of cervico-cerebral atherosclerosis and cerebral small vessel ischemia.