Plasma Aβ, homocysteine, and cognition

The Vitamin Intervention for Stroke Prevention (VISP) trial

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ABSTRACT

Background: Amyloid-beta protein (Aβ) plays a key role in Alzheimer disease (AD) and is also implicated in cerebral small vessel disease. Serum total homocysteine (tHcy) is a risk factor for small vessel disease and cognitive impairment and correlates with plasma Aβ levels. To determine whether this association results from a common pathophysiologic mechanism, we investigated whether vitamin supplementation–induced reduction of tHcy influences plasma Aβ levels in the Vitamin Intervention in Stroke Prevention (VISP) study.

Methods: Two groups of 150 patients treated with either the high-dose or low-dose formulation of pyridoxine, cobalamin, and folic acid in a randomized, double-blind fashion were selected among the participants in the VISP study without recurrent stroke during follow-up and in the highest 10% of the distribution for baseline tHcy levels. Concentrations of plasma Aβ with 40 (Aβ40) and 42 (Aβ42) amino acids were measured at baseline and at the 2-year visit.

Results: tHcy levels significantly decreased with vitamin supplementation in both groups. tHcy were strongly correlated with Aβ40 but not Aβ42 concentrations. There was no difference in the change in Aβ40, Aβ42 ($p = 0.40$, $p = 0.35$), or the Aβ42/Aβ40 ratio over time ($p = 0.86$) between treatment groups. Aβ measures were not associated with cognitive change.

Conclusions: This double-blind randomized controlled trial of vitamin therapy demonstrates a strong correlation between serum tHcy and plasma Aβ40 concentrations in subjects with ischemic stroke. Treatment with high dose vitamins does not, however, influence plasma levels of Aβ, despite their effect on lowering tHcy. Our results suggest that although tHcy is associated with plasma Aβ40, they may be regulated by independent mechanisms. *Neurology*® 2009;72:268–272

GLOSSARY

Aβ = amyloid-beta; **AD** = Alzheimer disease; **BMI** = body mass index; **DBP** = diastolic blood pressure; **MMSE** = Mini-Mental State Examination; **mRS** = modified Rankin Scale; **NIHSS** = NIH Stroke Scale; **SBP** = systolic blood pressure; **tHcy** = total homocysteine; **VISP** = Vitamin Intervention in Stroke Prevention study.

Amyloid β-protein (Aβ40, Aβ42) deposition in the brain is a hallmark of Alzheimer disease (AD) and is thought to be the cause of cognitive impairment and dementia.[1] Reduction of Aβ production is a candidate approach for treatment and prevention of cognitive impairment and dementia.[2] Plasma total homocysteine (tHcy) levels are correlated with plasma Aβ, although the biologic importance of this association is uncertain.[3-5] Plasma tHcy has been implicated as a risk factor for small vessel cerebrovascular disease and the development of cognitive impairment and dementia.[6-8]

There are several potential implications of these associations in relation to AD, cognitive impairment, and microangiopathy. tHcy may increase the risk of AD by elevating Aβ levels. Alternatively, Aβ may increase the risk of microangiopathic changes through elevation of tHcy

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levels. Neurotoxicity may be potentiated by the dual elevation of both tHcy and Aβ. Finally, tHcy and Aβ may be markers of a pathogenic mechanism and independent of each other.

Since plasma tHcy levels are readily modifiable by high-dose vitamin supplementation, we hypothesized that plasma Aβ levels may also be modifiable by vitamin supplementation. We thus aimed to test whether this association results from a common pathophysiologic mechanism between these biomarkers or if in fact they represent independent processes.

The Vitamin Intervention in Stroke Prevention (VISP) was a randomized controlled trial designed to test the hypothesis that lowering tHcy levels with large doses of folic acid, pyridoxine, and vitamin B_{12} would reduce the incidence of recurrent stroke or myocardial infarction.[9] Although the study did not show a benefit for the primary endpoint, tHcy was successfully lowered with vitamin therapy. We investigated plasma Aβ as an add-on component to the VISP study to test the hypotheses whether vitamin supplementation–induced reduction of tHcy over 2 years influences plasma Aβ levels.

METHODS

Subjects. Details of the VISP trial have been published previously.[9] Briefly, the VISP trial enrolled a total of 3,680 adults who 1) were within 120 days of a mild or moderate ischemic stroke (modified Rankin Scale [mRS] score of ≤ 3); 2) were 35 years or older; and 3) had a fasting tHcy level approximately greater than the 25th percentile for patients with stroke. Subjects were enrolled between September 1996 and December 2001 at 56 centers in the United States, Canada, and Scotland, and randomized to receive a high dose formulation (n = 1,827) containing 25 mg of pyridoxine, 0.4 mg of cobalamin (B_{12}), and 2.5 mg of folic acid or the low dose formulation (n = 1,853) of 200 mcg of pyridoxine, 6 mcg of cobalamin, and 20 mcg of folic acid.

Baseline VISP data included a standardized medical history, demographic variables, body mass index (BMI), stroke symptoms questionnaire, systolic and diastolic blood pressures, and a neurologic examination. Several scales which measure disability and cognition were administered to all patients (mRS, NIH Stroke Scale [NIHSS], Mini-Mental State Examination [MMSE]),[9] and serum levels of folate, B_{12} levels, and a lipid profile were ascertained. tHcy levels were obtained while fasting and after methionine loading.[9]

The assembly of the cohort for this substudy is shown in the figure. We selected at random a group of 150 patients treated with high dose formulation and another group of 150 patients with low dose formulation (within sex and 10-year age strata) among participants who did not have a recurrent stroke during the trial and who were in the highest 10% of the distribution for baseline tHcy levels, in order to maximize potential observed treatment effect. The sample numbers were selected based on power analysis and practical capacity for performing the outcome measures. In the current study, we had 85% power to detect an absolute difference of 7.5% in Aβ levels with high dose vitamin supplementation. Requirements for inclusion in the pool of potential subjects were availability of the following at both baseline and 2-year visits: blood samples, vitamin and tHcy levels, and MMSE. Individuals with less than 75% compliance by pill count were excluded. Plasma Aβ levels were measured in blood samples drawn at baseline and at the 2-year visit. This study was performed with approval by the ethics committees of all study institutions and administrative sites. Written informed consent was obtained from every potential participant. This substudy was performed with approval and in accord with the guidelines of our institutional review boards.

Blood collection. Blood was collected in polypropylene sterile plunger tubes containing potassium ethylenediamine tetraacetic acid. Samples were centrifuged at 1,380 *g* for 15 minutes, aliquoted with a protease inhibitor cocktail and frozen in dry ice, and stored at −80°C.

Biochemical assays. Plasma tHcy was determined by high-performance liquid chromatography, as detailed in our previous studies.[9-11] Plasma Aβ40 and Aβ42 concentrations were determined by sandwich ELISA using the BNT77 capture antibody and C-terminal specific detector antibodies BA27 and BC05 as previously described and validated.[4,12] We have demonstrated this ELISA system to detect Aβ40 or Aβ42 at concentrations as low as 1 pmol/L and to detect both free and protein-bound Aβ.[4,12] All biochemical analyses were performed without knowledge of subjects' clinical or radiographic information.

Statistical analyses. For univariate analysis, χ^2 tests were used to compare two categorical variables and analyses of variance were performed to compare continuous variables distributions across groups. All p values were two-tailed and criterion for significance was $p < 0.05$.

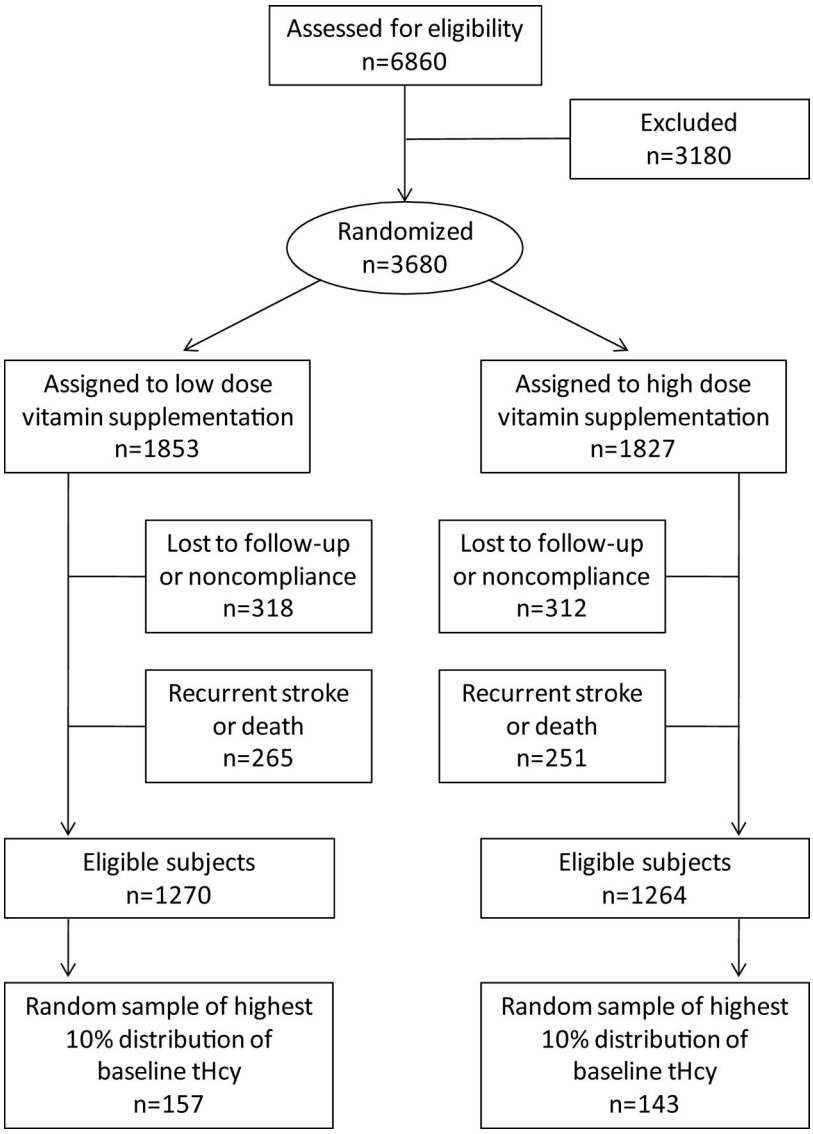
To determine whether vitamin treatment affected plasma Aβ levels, we adopted a linear mixed effects model for the data longitudinally measured over the 2-year period.[13] This technique allows for analysis of time-independent and time-dependent variables to identify associations with these variables as well as their trajectories over time. The parameter estimates indicate how much change in plasma Aβ levels resulted from a one unit change in each risk factor. Analyses were also performed using the Aβ42/Aβ40 ratio, as it has been recently demonstrated that this ratio may be associated with an increased risk of dementia.[14,15] For all models of the three outcome measures (Aβ40, Aβ42, and Aβ42/Aβ40 ratio), we investigated the effects of age, gender, clinical, and laboratory variables on change in plasma Aβ levels. Covariates that were associated with clinical scales in univariate analysis ($p < 0.10$) were considered in the final model.

RESULTS

The baseline clinical and demographic variables are presented in table 1. There were no significant differences in clinical or demographic variables between the two groups. There were no differences between Aβ40 or Aβ42 levels between treatment groups at baseline ($p = 0.97$ and 0.30, respectively). The median follow-up interval was 24.0 months.

Levels of tHcy at 2-year follow-up declined in both treatment groups (change in tHcy at 2 years

Figure Assembly of study sample from the Vitamin Intervention in Stroke Prevention (VISP) cohort



Recruitment for the VISP cohort is described in detail elsewhere.[9] Briefly, patients with a presumptive diagnosis of acute ischemic stroke were screened. Patients with total homocysteine levels (tHcy) that exceeded defined thresholds were randomized for treatment with high- or low-dose vitamin therapy. A random sample of eligible subjects with the highest 10% of tHcy were included in the study.

4.73 ± 8.98 in high treatment group and 1.66 ± 7.79 in the low treatment group; $p < 0.0001$ and $p = 0.009$, respectively). Reduction of tHcy was significantly greater in the high treatment group [β (time × treatment group) = −0.1289; $p < 0.0001$]. Baseline tHcy levels and tHcy levels at 2-year follow-up were significantly correlated with Aβ40 levels (r = 0.25 and 0.29, respectively; $p < 0.0001$ for both comparisons). However, tHcy levels were not correlated with Aβ42 levels at baseline ($p = 0.50$) or at 2-year follow-up ($p = 0.20$).

Levels of Aβ40, Aβ42, and the Aβ42-Aβ40 ratio at baseline and follow-up are shown in table 2. Aβ40 levels did not significantly change over the treatment period ($\beta = -0.09$, $p = 0.44$) and there was no significant difference in the change in Aβ40 levels between treatment groups ($\beta = 0.14$, $p = 0.40$). Similarly, there was no significant change in either Aβ42 levels or the Aβ42-Aβ40 ratio over time between treatment groups ($p = 0.35$ and $p = 0.86$) (table 2).

There was no association between Aβ40, Aβ42 levels, or the Aβ42/Aβ40 ratio with MMSE at baseline ($p = 0.10$, $p = 0.62$, and $p = 0.85$, respectively) or follow-up ($p = 0.28$, $p = 0.74$, and $p = 0.50$, respectively). Aβ levels did not influence change in MMSE over the treatment period.

DISCUSSION In this study, we sought to define the relationship between plasma Aβ levels and homocysteine lowering in a cohort of subjects from the randomized controlled VISP trial.[9] The current study demonstrates a strong association between tHcy and plasma Aβ40 levels in subjects with ischemic stroke in this longitudinal analysis. These findings confirm and extend cross-sectional observational studies which have previously reported this association.[3-5] However, despite the association of tHcy levels with Aβ40, Aβ40 levels were not influenced by vitamin treatment.

The strength of this study stems from the fact that subjects had randomized assignment to treatment type and that these subjects were followed prospectively for recurrent stroke and other cardiovascular events over a 2-year period with complete follow-up of all patients.

Elevated tHcy is a predictive factor for vascular disease, including ischemic heart disease and stroke.[16] Several studies have suggested that elevated tHcy is also a risk factor for white matter disease,[17] cognitive impairment,[17-20] and AD.[6-8] These associations may be explained by vascular[21] or direct neurotoxic[22,23] effects of tHcy.

Elevated plasma concentrations of Aβ are associated with microvascular disease in both population-based epidemiologic studies and cohorts of subjects with cognitive impairment.[4,24,25] In vitro studies have suggested direct physiologic or toxic effects of Aβ on the contractile/relaxation elements of the blood vessel wall.[26-28] Data regarding plasma Aβ and the risk of cognitive decline are conflicting. Cohort studies have reported that elevated plasma Aβ40 or Aβ42 levels increase the risk of developing AD over 5–8 years,[14,15,29] although a fourth found that plasma Aβ42 levels were not associated with cognitive decline over 30 months.[30] Other studies have found low Aβ40 or Aβ42 levels associated with incident AD[14,15] or more rapid cognitive decline in AD subjects.[31] Finally, some have suggested that low con-

Table 1 Baseline characteristics of subjects in cohort according to high or low vitamin treatment group

Characteristic	Low-dose group (n = 157), n (%)	High-dose group (n = 143), n (%)	p Value
Age, y	67.2 ± 10.2	66.7 ± 11.2	0.68
Sex			0.72
Male	88 (51.4)	93 (48.6)	
Female	55 (53.8)	64 (46.2)	
Current smoker	25 (55.6)	20 (44.4)	0.64
Ever smoked	99 (51.0)	95 (49.0)	0.54
BMI* (kg/m^2)	28.4 ± 5.4	29.6 ± 6.5	0.09
Homocysteine (μmol/L)	14.6 ± 5.7	15.7 ± 7.9	0.16
Vitamin B_{12} level	356.8 ± 213.8	369.3 ± 502.8	0.78
Total cholesterol	202.9 ± 42.6	203.3 ± 52.2	0.95
MMSE	27 ± 3	27 ± 3	0.99
mRS	1 (0, 2)	1 (0, 2)	0.36
NIHSS	0 (0, 1)	0 (0, 1)	0.77
SBP, mm Hg	141.5 ± 18.4	141.9 ± 20.4	0.85
DBP, mm Hg	78.2 ± 10.7	78.6 ± 9.9	0.71
Medication compliance,* %	98.55 ± 6.67	98.61 ± 7.43	0.94
Aβ40 (pmol/L)	72.5 ± 44.2	72.4 ± 39.3	0.98
Aβ42 (pmol/L)	18.3 ± 17.8	24.4 ± 69.9	0.33

Values are mean ± SD, median (25th, 75th quartile), or n (%).
*Measured at second follow-up visit.
BMI = body mass index; MMSE = Mini-Mental State Examination; mRS = modified Rankin scale; NIHSS = NIH Stroke Scale; SBP = systolic blood pressure; DBP = diastolic blood pressure.

centrations of plasma Aβ42 in combination with increased concentrations of plasma Aβ40 are associated with an increased risk of cognitive impairment and dementia.[14,15]

This study did not find a significant treatment effect of high dose vitamins on plasma levels of Aβ40 despite the effect of the high dose vitamins on lowering tHcy. This suggests that although tHcy is associated with plasma Aβ40, they may have independent pathophysiologic mechanisms. This is in contrast to Flicker et al.,[33] who detected an effect of tHcy lowering on plasma Aβ40. These differences may be reflective of the patient population (stroke patients with high tHcy in the VISP study, community dwelling older men in the study by Flicker et al.), confounding by dietary changes in folate consumption (VISP study), or differences in the form of Aβ measured by the assays (the assay used in this study measures protein bound Aβ, and does not detect oligomeric forms). Additionally, given the definition of the subcohort as those VISP subjects in the highest quintile of tHcy at baseline, a component of the reduction of tHcy may represent regression to the mean rather than vitamin effects.

Table 2 Linear mixed model analysis of plasma Aβ40, Aβ42, and Aβ42/Aβ40 and plasma tHcy at baseline and 2-year follow-up

Plasma biomarkers	Mean values at baseline		Mean values at 2-y follow-up		Treatment group × time p value
	Low-dose	High-dose	Low-dose	High-dose	
Aβ40 (μmol/L)	72.5 ± 44.2	72.4 ± 39.3	70.5 ± 43.61	72.8 ± 46.73	0.40
Aβ42 (μmol/L)	18.3 ± 17.8	24.4 ± 69.9	16.0 ± 21.50	25.9 ± 91.81	0.35
Aβ42/Aβ40	0.32 ± 0.35	0.46 ± 1.08	0.31 ± 0.63	0.37 ± 0.93	0.86
Homocysteine (μmol/L)	14.6 ± 5.7	15.7 ± 7.9	12.9 ± 5.3	11.0 ± 4.3	<0.0001

The VISP study results may suggest that tHcy is merely a marker for vascular disease and risk of cognitive decline because tHcy lowering does not influence Aβ40 levels.[9] Although tHcy is associated with plasma Aβ40, high dose vitamin treatment may differentially impact these two plasma markers.[4] Finally, correlations between tHcy and other metabolites of the methylation cycle, such as S-adenosylhomocysteine, have been reported.[34] How these metabolites respond to vitamin treatment remains to be elucidated. Further epidemiologic and therapeutic studies investigating the relationship between cerebrovascular disease and these potentially important plasma biomarkers (tHcy and Aβ40, Aβ42) are needed.

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