Plasma Total Homocysteine Concentrations in Epileptic Patients Taking Anticonvulsants

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Plasma total homocysteine (tHcy) and serum folate (FA) concentrations were measured in 130 epileptic patients taking anticonvulsant drugs. A significant inverse correlation was found between FA and tHcy. This was greater in the older group (≥15 years) than in the younger group (1 to 14 years). There were four FA-deficient patients (FA concentration <3 ng/mL regardless of symptoms), including three patients in the older group and one in the younger group. All FA-deficient patients had received long-term treatment (>7 years) with multiple anticonvulsants. Their tHcy levels were higher than the 90th percentile of those in control subjects. Two patients showed extremely high levels of tHcy (57.9 and 29.1 μmol/L) and subnormal plasma methionine levels. After FA therapy, their tHcy decreased to levels the same as or less than those of control subjects and FA increased to above the normal range. Based on these findings, we conclude that measuring FA and tHcy concentrations may be useful for preventing thrombosis due to hyperhomocysteinemia in epileptic patients taking anticonvulsants, particularly those who receive long-term treatment with multiple agents.

IT IS WELL KNOWN that folate (FA) deficiency occurs in some epileptic patients taking anticonvulsant drugs.[1] There is a high incidence of neuropsychiatric illness in epileptic patients with megaloblastic anemia due to FA deficiency.[1]

Although the mechanisms by which anticonvulsants induce FA deficiency are unclear, the proposed mechanisms can be summarized as (1) interference with the intestinal absorption of FAs, (2) induction of enzymes in the liver that require and finally deplete FA, and (3) interference with the metabolism of FA coenzymes.[1]

FA deficiency is a common condition that is usually caused by low dietary FA intake or interference of FA metabolism by drugs, such as anticonvulsants.[2] FA is required for remethylation of homocysteine to methionine in sulfur-containing amino acid metabolism. It is therefore conceivable that FA deficiency may cause hyperhomocysteinemia, which might be a risk factor for arteriosclerosis.[2] Several investigators have reported that FA deficiency induces hyperhomocysteinemia, but that oral treatment with high doses of FA corrects elevated plasma total homocysteine ([tHcy] protein-bound and free homocysteine) concentrations in such patients within a few days.[3,4] However, it remains undetermined to what degree FA deficiency influences the metabolism of homocysteine in epileptic patients. It is also unclear what percentage of FA-deficient epileptic patients should be treated.

The metabolism of homocysteine to cystathionine is catalyzed by a vitamin B6–dependent enzyme, ie, cystathionine β-synthase. Since Ubbink et al[5] reported that differences in fasting tHcy concentrations between vitamin B_6–deficient patients and controls were not statistically significant, in this study we did not measure plasma vitamin B_6 concentrations.

Vitamin B_{12} is another nutritional factor required for remethylation of homocysteine to methionine, and a deficiency induces hyperhomocysteinemia.[2]

In this study, concentrations of plasma tHcy, serum FA and vitamin B_{12} were measured in epileptic patients receiving anticonvulsants. The relationship between FA and tHcy concentrations was examined. In patients with hyperhomocysteinemia and FA deficiency, tHcy and FA were measured again after FA therapy. These analyses showed that hyperhomocysteinemia due to FA deficiency occurs in patients receiving multiple drugs and long-term anticonvulsant therapy, and that FA supplementation reduces tHcy levels.

SUBJECTS AND METHODS

One hundred thirty epileptic outpatients regularly evaluated at the neurologic clinic of the Department of Pediatrics, Hiroshima University School of Medicine, were enrolled onto the study. The age range was 2 to 35 years (mean, 14.3). There were 100 patients undergoing anticonvulsant monotherapy and 30 undergoing multiple-drug therapy. Agents in the former group consisted of valproic acid (42 cases), carbamazepine (34 cases), phenobarbital (11 cases), phenytoin (10 cases), primidone (one case), and zonisamide (two cases). Agents in the latter group consisted of combinations of these drugs. The mean duration of treatment was 5.9 years (ranges, 1 to 24). A treatment duration longer than 5 years was defined as long-term therapy and less than 5 years as short-term therapy. There were no patients with malnutrition or who were receiving FA or other vitamins. Liver and renal function was normal in all patients. The controls consisted of 81 healthy subjects in whom tHcy concentrations were measured. Their ages ranged from 1 to 33 years (mean, 15.1). Informed consent was obtained from all subjects before enrollment onto the study.

Blood samples in epileptic patients were obtained a few hours after they took anticonvulsants in the morning, and those in control subjects were also obtained during the morning. All samples were nonfasting, since there were no significant differences in tHcy values of six healthy adults before and after breakfast (4 hours later) in our preliminary experiment (data not shown).

Samples of venous blood were drawn, and the plasma and serum were immediately separated (800 × *g* for 10 minutes). tHcy concentrations in epileptic patients and control subjects were measured as follows using a modified method of Cornwell et al.[6] To 20 μL plasma, 80 μL 2.5-mmol/L EDTA-Na_2 was added. To 20 μL diluted plasma, 20 μL 4-mmol/L dithioerythritol (in 0.05 mol/L Tris, 5 mmol/L EDTA-Na_2,

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and 1 mol/L Na_2SO_4, pH 9.0) was added. This mixture was incubated at 37°C for 10 minutes. After reduction, 60 μL ABD-F (4-aminosulfonyl-7-fluoro-2,1,3-benzoxa-diazole 0.2 mg/mL in 0.2 mol/L Tris and 50 mmol/L sodium arsenite, pH 8.0) was added to the mixture. A 20-minute incubation at 60°C was used to derivatize a fluorescent compound. A 100-μL aliquot of 0.3N $HClO_4$ was added to the mixture to stop the reaction. The samples were centrifuged at 3,500 × *g*.

The clear supernatant was filtered through a Cosmonice Filter W (Nakarai Tesque, Kyoto, Japan) and used for high-performance liquid chromatographic (HPLC) analysis. The HPLC system was equipped with an LC-9A pump (Shimadzu, Kyoto, Japan), an RF-535 fluorescence spectrophotometer (Shimadzu), an ODS-80 A column (6.0 × 150 mm; GL Science, Kyoto, Japan), and a CTO-6A column oven (Shimadzu). The fluorescence spectrophotometer was operated at an excitation wavelength of 375 nm and an emission wavelength of 515 nm. The mobile phase, pumped at 1.0 mL/min, consisted of 0.1 mol/L sodium dihydrogenphosphate (pH 4.5) containing 4.8% acetonitrile. The temperature of the column oven was set at 40°C. Peak areas were measured by a C-R4A recorder-integrater (Shimadzu). Using this method, the interassay coefficient of variation was 4.9%. FA and vitamin B_{12} concentrations were measured using a competitive protein-binding radioassay using vitamin B_{12}/FA kits purchased from Ciba-Corning Diagnostics (Medfield, MA). Plasma amino acids, including methionine and homocystine, were determined with an amino acid autoanalyzer (Hitachi).

The data are presented as the median and the range from the 25th to 75th percentile. The Mann-Whitney *U* test, Spearman rank test, and Fisher's exact probability test were used to analyze the data.

RESULTS

Since it has been reported that FA concentrations decrease with age in normal subjects[7] (Table 1), control subjects were grouped according to age for tHcy measurements as follows: (1) 1 to 4 years, (2) 5 to 9 years, (3) 10 to 14 years, and (4) 15 years and older (mean age, 24.3 years; range, 15 to 33). There were no significant differences among the three groups of subjects younger than 15 years (mean age, 6.6 years; range, 1 to 14). Thus, for the purpose of this study, they were treated as one group. However, tHcy levels in the group aged 15 years and older were significantly higher than those in the group aged 1 to 14 years (Table 2). Therefore, epileptic patients were divided into two age groups: 1 to 14 years (mean age, 9.2 years; range, 1 to 14) and 15 years and older (mean age, 21.2 years; range, 15 to 35).

Relationship Between FA and tHcy Concentrations

Significant inverse correlations were found between FA and tHcy concentrations in both the younger and older groups ($r = -.289$ and $-.465$, respectively; Figs 1 and 2). The correlation coefficient was greater in the older group than in the younger group.

Table 1. Reference Values for FA in Children

Age Group (yr)	No.	FA (ng/mL) Mean	FA (ng/mL) Range
1-4	27	6.38	3.2-12.3
5-9	13	5.32	3.0-8.0
10-14	19	4.46	3.0-5.3
≥15	15	3.62	2.5-4.8

Table 2. tHcy in Control Subjects

Age Group (yr)	No.	tHcy (μmol/L) Mean	tHcy (μmol/L) Range
1-4	17	4.0	3.3-6.2*
5-9	13	3.6	2.7-4.3*
10-14	12	3.9	3.2-5.8*
1-14	42	3.8	3.2-5.7*
≥15	39	6.3	5.1-9.4

*$P < .01$ *v* group aged ≥15 years (Mann-Whitney *U* test).

Fraction of FA-Deficient Patients

Kishi et al[7] have reported that the low-normal FA value ranges from 2.5 to 3.2 ng/mL and is independent of age. Since FA in the present study was measured in the same way, patients who had a FA concentration less than 3 ng/mL were considered FA-deficient regardless of symptoms.

There were no cases of FA deficiency identified in subjects in the younger or older monotherapy groups (Table 3). However, of the patients undergoing multiple drug therapy, one FA-deficient patient was found in the younger group (7.7%) and three in the older group (17.6%) (Table 3).

FA-Deficient Patients

All four patients with FA deficiency had received long-term treatment with multiple anticonvulsants. FA levels in these patients ranged from 2.4 to 2.9 ng/mL (Table 4). There were no patients with associated anemia or neuropsychiatric illness. In addition, their tHcy levels were higher than the 90th percentile of those in the control subjects (7.6 μmol/L in the younger group and 10.5 μmol/L in the older group). Two patients showed markedly elevated tHcy levels; the value in patient no. 3 was 57.9 μmol/L (the highest value among all patients) and in patient no. 4 29.1 μmol/L. Their plasma methionine levels were

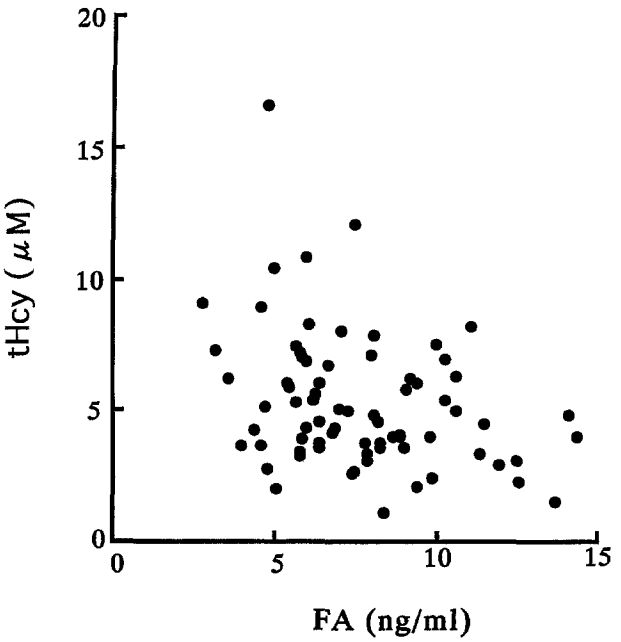

Fig 1. Correlation between FA and tHcy for group aged 1 to 14 years. $r = -.289$, $P < .05$ (Spearman rank test).

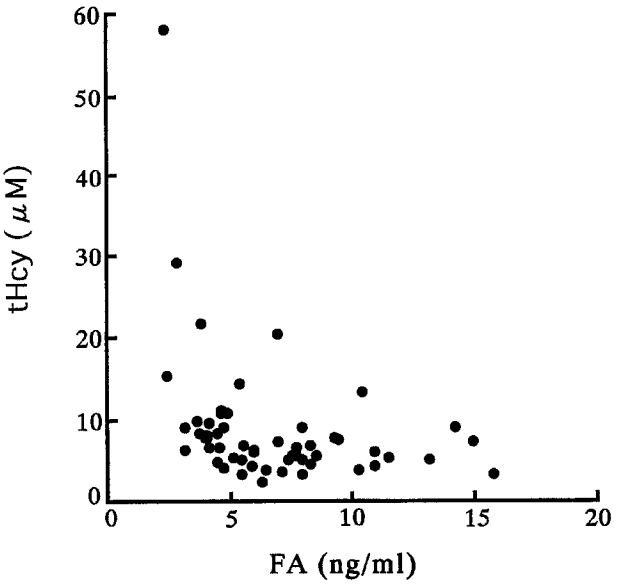

Fig 2. Correlation between FA and tHcy for group aged ≥15 years. $r = -.465$, $P < .001$ (Spearman rank test).

subnormal. However, their homocystine levels were within the normal range despite the extremely high levels of tHcy.

After oral treatment with FA (10 mg/d in patient no. 3 and 20 mg/d in patient no. 4), their tHcy decreased to levels the same as or less than those of the control subjects and FA increased to a much higher level than the normal range (Table 5). FA therapy resulted in a cessation of seizures in patient no. 3.

Serum Vitamin B_{12} Levels in Epileptic Patients

Serum vitamin B_{12} levels were within the normal range in all patients (230 to 1,200 pg/mL). There was no correlation between vitamin B_{12} and tHcy concentrations.

DISCUSSION

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. FA and vitamin B_{12} are required for remethylation of homocysteine to methionine. 5-Methyltetrahydrofolate serves as the methyl donor for this reaction and vitamin B_{12} as the coenzyme. A deficiency of these factors will result in impaired remethylation, methionine depletion, and accumulation of homocysteine, which produces atheromatous changes.[2]

Table 3. Percentage of FA-Deficient Patients

	Age Group			
	1-14 Years		≥15 Years	
Therapy	No.	%	No.	%
Monodrug	0/62	0	0/38	0
Multidrug	1/13	7.7	3/17	17.6*
All	1/75	1.3	3/55	5.5

NOTE. The number of FA-deficient patients is indicated in the numerator, and the number of all patients in each group is indicated in the denominator.

*$P < .05$ *v* group aged ≥15 years treated with monodrug therapy (Fisher's exact probability test).

Table 4. Clinical Characteristics of the FA-Deficient Patients

	Case No.			
Characteristic	1	2	3	4
Age (yr)	11	30	20	20
Sex	M	F	M	F
Drug treatment	PRM (7)	PB (13)	PB (19)	PB (7), PHT (11)
	VPA (9)	CBZ (13)	PRM (19)	VPA (7), CBZ (2)
			PHT (19)	CZP (7), ZNS (5)
FA (ng/mL)	2.8	2.5	2.4	2.9
tHcy (μmol/L)	9.1	15.3	57.9	29.1
HM (μmol/L)	—	—	ND	Trace
Met (μmol/L)	—	—	13.5	15.4

NOTE. The normal range for HM is trace to 2.6, and for Met, 19.4 to 39.5. Years of drug administration are indicated in parentheses.

Abbreviations: HM, plasma homocystine concentration; Met, plasma methionine concentration; PRM, primidone; VPA, valproate; PB, phenobarbital; CBZ, carbamazepine; PHT, phenytoin; CZP, clonazepam; ZNS, zonisamide; ND, not detected.

This study demonstrated that there was a significant inverse correlation between FA and tHcy concentrations in epileptic patients taking anticonvulsant drugs. However, there was no such correlation between vitamin B_{12} and tHcy. Since hyperhomocysteinemic patients with low serum FA (patients no. 3 and 4) showed low plasma methionine levels and vitamin B_{12} levels within the normal range, it was suspected that the hyperhomocysteinemia was due to FA deficiency. After FA therapy, their tHcy decreased to levels the same as less than those in control subjects, and their FA increased to a much higher level than the normal range. These findings suggest that FA deficiency induced hyperhomocysteinemia in these epileptic patients treated with anticonvulsants.

The significant inverse correlation between FA and tHcy was greater in the older group than in the younger group. Several investigators have reported that the low FA levels are associated with the duration of anticonvulsant therapy.[1,8] Most patients in the older group had been treated since childhood, during which their first seizures occurred. In fact, in the fraction of patients treated with long-term anticonvulsant therapy, there were significantly more patients in the older group than in the younger group (63.5% *v* 27.8%, $P < .01$ by Fisher's exact probability test). Kang et al[3] have reported that some patients receiving FA had high serum FA concentrations and high levels of protein-bound homocysteine. Kang et al[3] and Ueland and Refsum[2] speculated that this might be related to tissue depletion of reduced FA before FA administration, and thus the plasma homocysteine concentration probably reflected the intracellular FA status. Based on this view, we hypothesize that the persistent low levels of serum FA in older patients who had undergone long-term therapy are a reflection of low tissue FA concentra-

Table 5. Changes in FA and tHcy in Patients With Hyperhomocysteinemia Following FA Administration

		tHcy (μmol/L)		FA (ng/mL)	
Case No.	Treatment Period	Before	After	Before	After
3	9 mo	57.9	5.6	2.4	95
4	2 mo	29.1	2.8	2.9	47.9
Normal range		5.1-8.4		3.1-8.3	

tions. As a result, a much more significant inverse correlation between FA and tHcy concentrations was found in the older group compared with the younger group. This hypothesis might explain the difference in tHcy levels among FA-deficient patients (the older patients, no. 2 to 4, had higher levels of tHcy than the younger patient, no. 1, although their FA levels were almost the same).

It is unclear why FA deficiency due to multiple-agent long-term anticonvulsant therapy induced extreme hyperhomocysteinemia, as in patients no. 3 and 4. As previously described, it was suspected that the hyperhomocysteinemia was induced by FA deficiency itself or FA deficiency due to decreased enzyme activity in FA metabolism, such as 5,10-methylenetetrahydrofolate reductase (MTR). MTR supplies 5-methyltetrahydrofolate, the coenzyme for methionine synthase (MS), which is involved in the remethylation of homocysteine. Since in these patients plasma methionine concentrations were low, MS activities might also be decreased. In some studies using animals, MTR activity was decreased[9] or increased[10] and MS activity was unaltered[10] or increased[11] during phenytoin treatment. Thus, the subject of these enzyme activities in anticonvulsant treatment has been controversial. To clarify the relation of the activities of MTR or MS and hyperhomocysteinemia in epileptic patients, measurement of the enzyme activities will be required in the future.

In recent studies, mild to moderate homocysteinemia has been demonstrated to be a risk factor for vascular disease.[2,12,13] Stampfer et al[12] reported that men with tHcy levels above the 95th percentile had a threefold increased risk of myocardial infarction. It was also reported that the risk for coronary artery disease was increased with a 5-μmol/L increase in tHcy levels.[14] For these reasons, administration of FA would be recommended in epileptic patients with hyperhomocysteinemia due to FA deficiency to prevent vascular disease.

Mudd et al[15] have reported that high intraperitoneal doses of DL-homocysteine induced tonic-clonic grand mal seizures in rats. Marangos et al[16] reported that homocysteine elicited seizures in mice at a dose of 850 mg/kg (95% to 100% of animals) and that the homocysteine levels used in their study were in the range of those observed in the serum of homocysteinuric patients (100 to 200 μmol/L). It has also been reported that approximately 21% of homocystinuric patients with cystathionine β-synthase deficiency not treated from early infancy have suffered seizures.[17] Since in our study there were no patients who showed the same levels of plasma tHcy as homocystinuric patients, it is unclear whether their seizures were induced by hyperhomocysteinemia. However, from the fact that FA therapy resulted in cessation of seizures in patient no. 3, hyperhomocysteinemia might have some influence on seizures in this patient. Further study will be needed to clarify this matter because of the small number of hyperhomocysteinemic patients in our study.

In conclusion, measuring FA and tHcy concentrations may be beneficial in the treatment of epileptic patients taking anticonvulsant drugs, particularly those undergoing multiagent and/or long-term anticonvulsant therapy.

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