Epilepsy Research (2012) **102**, 1—7

journal homepage: www.elsevier.com/locate/epilepsyres

REVIEW

Antiepileptic drugs, hyperhomocysteinemia and B-vitamins supplementation in patients with epilepsy

Vincenzo Belcastro[a,*], **Pasquale Striano**[b]

[a] *Neurology Clinic, Department of Neuroscience, Sant'Anna Hospital, Como, Italy*
[b] *Pediatric Neurology and Muscular Diseases Unit, G. Gaslini Institute and University of Genova, Genova, Italy*

Received 1 April 2012; received in revised form 20 June 2012; accepted 3 July 2012
Available online 21 July 2012

KEYWORDS
Homocysteine;
Folic acid;
B-vitamins;
Antiepileptic drugs;
Epilepsy

Summary Homocysteine (Hcy) is a sulfur-containing, nonprotein amino acid reversibly formed and secreted during metabolism of methionine. Elevated total Hcy levels (hyper-tHcy) have been associated with cardiovascular disease in multiple large-scale epidemiologic studies and, in particular, patients with epilepsy exhibit elevated plasma tHcy levels more frequently than the general population caused by polymorphisms in the *MTHFR* gene and chronic treatment with older antiepileptic drugs.

Folic acid alone or folic acid combined with other B-vitamins have all been shown to reduce tHcy concentration in patients on chronic treatment with antiepileptic drugs, however, which is the most appropriate supplementation scheme of folic acid and/or B-vitamins in patients with epilepsy still remains matter of debate. We review the latest findings on the role of supra-physiological tHcy concentrations as vascular risk factor in patients with epilepsy and discuss the possible role played by folate and other B-vitamins supplementation in epileptic patient with hyper-tHcy.

Contents

* Corresponding author at: Tel.: +39 0 31 5859953.
E-mail address: vincenzobelcastro@libero.it (V. Belcastro).

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http://dx.doi.org/10.1016/j.eplepsyres.2012.07.003

Introduction

The interest of the medical community in the amino acid homocysteine (Hcy) stems from the observation that elevated total serum Hcy (tHcy) has been associated with cardiovascular disease in multiple large-scale epidemiologic studies. Thus, hyper-tHcy has been considered as an independent risk factor for atherosclerosis and stroke (Hankey and Eikelboom, 1999, 2001; Spence, 2007). Moreover, several epidemiological studies and clinical investigations, have associated hyper-Hcy to epilepsy, Parkinson's disease, Alzheimer's disease and multiple sclerosis and experimental data have provided further support to this hypothesis, having evidenced that Hcy may induce cell damage through a number of complex mechanisms, including formation of reactive oxygen species, increased lipid peroxidation, induced nitrosative stress and impaired production of the antioxidant glutathione peroxidise (Ientile et al., 2010).

Noteworthy, patients with epilepsy exhibit elevated plasma tHcy levels more frequently than the general population (10—40% vs. 5%) due to (1) the reduced activity of the key enzyme 5,10 methylenetetrahydrofolate-reductase (MTHFR) caused by polymorphisms in the MTHFR gene and (2) a deficiency of folate, an important cofactor in the metabolism of Hcy, induced by older antiepileptic drugs (AEDs), such as carbamazepine, phenytoin, phenobarbital and primidone (Verrotti et al., 2000; Apeland et al., 2001; Reynolds, 2006; Belcastro et al., 2007; Mintzer et al., 2009; Belcastro et al., 2010; Linnebank et al., 2011; Bochyńska et al., 2012; Coppola et al., 2012). Interestingly, hyper-tHcy in epileptic patients has been suggested to contribute to the development of atherosclerosis (Elliott et al., 2007; Hamed et al., 2007; Chuang et al., 2012), brain atrophy (Gorgone et al., 2009), and seizure aggravation, as also supported by experimental data on animals (Folbergrová et al., 1997; Baldelli et al., 2010). Since older AEDs treatment constantly deplete the organism of folate through induction of the hepatic enzymes, the benefit of folate intake has been proposed to provide benefit in preventing cardiovascular events due to high levels of tHcy in patients with epilepsy (Belcastro et al., 2007; Mintzer et al., 2009; Belcastro et al., 2010; Linnebank et al., 2011).

We review the latest findings on the role of supraphysiological tHcy concentrations as vascular risk factor in patients with epilepsy and discuss the possible role played by folate and other B-vitamins supplementation in epileptic patient with hyper-tHcy (Table 1).

Homocysteine metabolism

Homocysteine is a sulfur-containing, nonprotein amino acid reversibly formed and secreted during metabolism of methionine. Once formed, Hcy is metabolized via two pathways: (i) re-methylation to methionine, which requires methylenetetrahydrofolate reductase (MTHFR)/methionine synthase (MS) or betaine homocysteine methyltransferase (BHMT), and folic acid and vitamin B12 as co-factors; (ii) trans-sulfuration to cysteine, which requires cystathionine-beta-synthase (CBS) and pyridoxal-5′-phosphate, the vitamin B6 coenzyme (Mattson and Shea, 2003). Hcy represents, through methionine metabolism, a key intermediate in the methylation pathway, which provides one-carbon methyl groups for transmethylation reactions. These are widely involved in several biological processes (Mattson and Shea, 2003; Perla-Kajàn et al., 2007). The activated S-adenosyl methionine (SAM) is required for several transmethylation reactions involving different substrates such as phospholipids, myelin, choline and cathecolamines. S-adenosyl-methionine generated in these reactions is in its turn hydrolyzed to Hcy and adenosine by S-adenosylhomocysteine hydrolase. Hcy is then re-methylated to methionine by remethylaton pahway, involving MS and BHMT, and/or converted to cystathionine by thans-sulphuration pathway involving CBS (Ientile et al., 2010).

Homocysteine is present in plasma in four forms: about 1% circulates as the free thiol; 70—80% is disulphide-bound to plasma proteins, chiefly albumin; and the remaining 20—30% combines with itself to form the dimer homocysteine or with other thiols, including cysteine, with which it forms the homocysteinecysteine mixed disulphide (Hankey and Eikelboom, 1999). The term ''total plasma (or serum) homocysteine'' (tHcy) refers to the combined pool of all four forms of homocysteine. An abnormal tHcy is defined by an arbitrary cut-off (e.g., 95th percentile) in the distribution of concentrations found in the ''normal population''. Among fasting individuals, ''normal'' tHcy commonly ranges from 5 to 15 μmol/L, and higher fasting values are classified arbitrarily as moderate (16—30), intermediate (31—100), and severe (>100 μmol/L) hyper-tHcy (Hankey and Eikelboom, 1999).

Genetic polymorphisms involved in homocysteine metabolism

Changes in the activity and availability of enzymes involved in the regulation of Hcy levels may be important in the regulation of the plasma tHcy levels and hence phenotype of complex diseases.

Polymorphisms in the genes involved in the methionine and the folate cycles and alterations of the trans-sulfuration pathway may induce elevation of plasma tHcy levels. The alterations involved in the quantitative changes of Hcy metabolism are sequence repeat and single nucleotide polymorphism at genetic level and epigenetic modifications.

5,10-methylene-tetrahydrofolate reductase (MTHFR) gene polymorphisms are characterized by a base substi-

Table 1 Supplementation trials with folic acid and B-vitamins in patients with epilepsy.

Authors	Patients (*n*)	Folate (mg per day)	B-vitamins (per day)	Supplementation (day)
Apeland et al. (2002)	33	0.4 mg	B6 120 mg B2 75 mg	30
Belcastro et al. (2007)	59	5 mg	no	30
Bochyńska et al. (2012)	20	0.4 mg	B6 50 mg B12 100 μg	365
Huemer et al. (2005)	19	1 mg	no	90
Linnebank et al. (2011)	141	5 mg	B12 900 μg	90

tution from C to T on residue 677 and A to C on residue 1298. In particular, the T677 variant of MTHFR gene is associated with reduced enzyme activity in vitro (Rozen, 1997; Weisberg et al., 1998). MTHFR gene C677T and A1298C polymorphisms are common in the general population (Chango et al., 2000). The T677 allele occurs in 35% of Caucasian populations and TT homozygotes may achieve a percentage of 10—20%. Carriers of the TT677 MTHFR genotype frequently exhibit elevated plasma Hcy, especially if stores of folate or vitamin B12 are depleted. The TT mutation has been described to result in reduction of the MTHFR enzymatic activity >50% (Chango et al., 2000; Mattson and Shea, 2003; Ientile et al., 2010). The A1298C polymorphism alone does not affect appreciably plasma Hcy but it may do so when combined with the 677T variant (AC/CT) (Weisberg et al., 2001).

The first enzyme in trans-sulfuration pathway, CBS, is a B6—dependent heme protein in mammals. Common mutations in the gene (G919A and T833C) may lead to hyper-tHcy (Tsai et al., 1996). In heterozygous carrier state of CBS mutations, plasma tHcy levels can be normal in basal conditions, but they become abnormally high after an oral methionine loading test (Guttormsen et al., 2001). Patients with homozygous homocystinuria have a severe deficiency of CBS activity and exhibit very high (>100 μmol/L) plasma tHcy levels. Furthermore, Sniezawska et al. reported polymorphisms of MTHFD1GG (G1958A) related to increased tHcy levels in epileptic patients (Sniezawska et al., 2011).

Antiepileptic drugs, mthfr, b-vitamins and hyperhomocysteinemia

Epileptic patients exhibit, in a percentage of 20—40%, supraphysiological plasma levels of tHcy as a consequence of the interplay between variants of the MTHFR gene polymorphisms (Caccamo et al., 2004; Belcastro et al., 2007) and the chronic intake of older AEDs (Reynolds, 2006; Mintzer et al., 2009; Belcastro et al., 2010; Linnebank et al., 2011). Several studies have suggested that treatment with distinct AEDs like carbamazepine, phenobarbital, primidone and phenytoin is associated with reduced mean serum levels of folate and vitamin B12 (Apeland et al., 2001; Belcastro et al., 2007; Schwaninger et al., 1999; Verrotti et al., 2000) and that this may mediate AED side effects (Kishi et al., 1997; Reynolds, 1968, 2006). Concerning data on vitamin B6 blood levels, conflicting findings exist in literature. In fact, a recent study shows that vitamin B6 blood levels are not influenced by AED treatment (Linnebank et al., 2012) whereas Mintzer et al. found that B6 deficiency is very common in the inducer AEDs-treated population (Mintzer et al., 2012).

Conventional AEDs have a high liver enzymes inducer potential and are thought to determine elevated tHcy levels by increasing catabolism and elimination of folate, which serve as cofactors/substrates in Hcy metabolism (Schwaninger et al., 1999). Although folate deficiency is considered to be potentially less common in patients on new AEDs due to their overall lower enzyme-inducing activity (Reynolds, 2006), treatment with oxcarbazepine and topiramate has been additionally associated with higher tHcy plasma levels (Belcastro et al., 2010; Linnebank et al., 2011). Conversely, lamotrigine and levetiracetam are devoid of this effect (Gidal et al., 2005; Mintzer et al., 2009; Belcastro et al., 2010).

The C677T is the most common MTHFR polymorphism that, in the homozygous state, implies a 50—60% decrease in the enzymatic activity and low folate levels (Chango et al., 2000). Moreover, in a previous study we have observed a more frequent association between MTHFR gene C677T and A1298C polymorphisms in epileptic patients than in the control sample (Caccamo et al., 2004). Carriers of AC/CT combination and concomitant low folate concentrations exhibited the highest tHcy plasma levels in patients with epilepsy (Caccamo et al., 2004). Thus, interplay of enzyme inducing AEDs and MTHFR polymorphisms causes low plasma folate and hyper-tHcy (Apeland et al., 2002; Caccamo et al., 2004; Belcastro et al., 2007, 2010) and affects the reformation rate of hyper-tHcy once folate is discontinued (Belcastro et al., 2007). For these reasons, patients on chronic AEDs treatment exhibit a greater risk for hyper-tHcy than the general population. Of note is that seizures in many patients do not remit despite appropriate medication, and lifelong AED therapy is usually required for those with refractory epilepsy (Kwan and Brodie, 2000).

Hyperhomocysteinemia in epileptic patients: how to assess the clinical risk?

The effect of AEDs on the cardiovascular system is a relevant research area due to the increased atherosclerotic risk in subjects receiving chronic therapy for epilepsy (Belcastro and Striano, 2012). Among the various variables analyzed, hyper-tHcy have been indicated as a risk factor for the progression of atherosclerosis in epileptic patients (Hamed and Nabeshima, 2005; Elliott et al., 2007; Hamed et al., 2007; Tan et al., 2009; Chuang et al., 2012).

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide (NO) synthase inhibitor and increased plasma ADMA levels have been associated with cardiovascular morbidity (Vallance, 2001). Hyper-tHcy has been linked to elevated ADMA levels, which are factors that may be

better indicators of endothelial dysfunction compared to tHcy levels, because they are less sensitive to changes, such as fasting status, physical activity, and other factors (Oz et al., 2009). Interestingly, elevated ADMA levels have been claimed as responsible for the increased cardiovascular risk in patients with epilepsy under carbamazepine and valproate therapy (Oz et al., 2009; Ozdemir et al., 2011). Noteworthy, literature data suggest that patients with epilepsy exhibit an increased risk for stroke (Cockerell et al., 1994; Cleary et al., 2004). In this sense, definition of a standardized clinical assessment is essential to develop new therapeutic strategies, including vitamin supplements, as well as to reduce morbidity and mortality in epilepsy patients under long-term therapy.

Noninvasive measurement of intima media thickness (IMT) at the far wall of the common carotid artery (CCA) by high resolution B-mode ultrasound is widely used in observational studies and validated as a surrogate marker for early prediction of atherosclerosis (Polak et al., 2011). Interestingly, several studies suggest that changes in various vascular risk factors including hyper-tHcy and increase in carotid IMT may be attributed to AEDs (Hamed et al., 2007; Tan et al., 2009; Chuang et al., 2012). Thus, because of atherosclerosis progresses slowly over time and the relationship between the duration of AED therapy and atherosclerotic risk has been claimed, measurement of IMT of the CCA could represent a good indicator of atherosclerosis in epilepsy patients (Hamed et al., 2007). Of note is that prolonged treatment with valproate raises tHcy levels in epileptic children (Verrotti et al., 2000; Karabiber et al., 2003; Attilakos et al., 2006) and an increased in CCA-IMT has been showed in epileptic children treated with valproate (Erdemir et al., 2009).

Retinal vessels share anatomic and functional properties with small cerebral arteries and their assessment provides a unique and noninvasive method for investigating subclinical cerebral microangiopathy (Mitchell et al., 2005). Although data from a meta-analysis suggest that hyper-tHcy is a risk factor for retinal vascular occlusive disease (Cahill et al., 2003), recently, we failed to identify precocious retinal vascular caliber change in epileptic patients with hyper-tHcy by retinal photographic examination (Belcastro et al., 2008, 2009).

The ankle brachial index (ABI) is an indicator of lower extremity peripheral arterial disease (PAD) and a predictor of atherothrombosis (Hirsch et al., 2006). Interestingly, a recent meta-analysis concluded that patients with PAD have an elevated blood level of tHcy (Khandanpour et al., 2009). Despite the lack of firm knowledge of mechanisms underlying the association between hyper-tHcy and PAD, a recent study suggests that tHcy could exert deleterious effects on peripheral arteries by influencing ApoA-I and the HDL-dependent transport of cholesterol (Guéant-Rodriguez et al., 2011).

Hyperhomocysteinemia and b-vitamins supplementation in patients with epilepsy

Folic acid supplementation has been a topic of discussion within the epilepsy community for several decades because of folic acid was suspected to be epileptogenic (Reynolds, 1968, 1973; Morrell, 2002). However, recent research has demonstrated that folic acid does not promote seizures (Reynolds, 2006).

Since a condition of hyper-tHcy would be easily corrected by intake of folate and other B vitamins, how should folic acid and/or B-vitamins be provided in patients with epilepsy?

Concerning folate dose alone to be prescribed, no unanimous consensus exists among authors and a wide range from <0.4 to 5 mg daily can be found in the literature (Apeland et al., 2002; Huemer et al., 2005; Belcastro et al., 2007; Harden et al., 2009; Linnebank et al., 2011). In a previous study, we used a daily folate dose of 5 mg for 30 days which was sufficient to normalize a condition of moderate to intermediate hyper-tHcy (Belcastro et al., 2007). However, six months after folate discontinuation 73% of patients taking enzyme-inducing (EI) AEDs reformed hyper-tHcy (Belcastro et al., 2007). Moreover, we found that the time course of hyper-tHcy recurrence was different among groups, being more rapid in the MTHFR 677TT/1298AA-patients treated with older EI AEDs and slower in the MTHFR677CT/1298AA group (Belcastro et al., 2007).

In another large monocenter study, patients on chronic treatment con EI-AEDs showed folate or vitamin B12 levels below the reference range which were associated with higher mean corpuscular volume (MCV) and higher tHcy levels (Linnebank et al., 2011). In this study, vitamin substitution for 3 months with folate 5 mg/day or vitamin B12 900 μg/day or with both restored the normal vitamin levels in 95% of the supplemented patients and reduced MCV and tHcy plasma levels (Linnebank et al., 2011). Interestingly, the effects of different B-vitamins, other than folic acid and cobalamin, have been used to restore elevated tHcy levels in patients taking AEDs (Apeland et al., 2002). In this study, subjects were supplemented for 30 day with folic acid 0.4 mg, pyridoxine (i.e. B6) 120 mg and riboflavin (i.e. B2) 75 mg per day. These vitamins given for 1 month normalize the efficiency of the remethylation pathway and partially improve the transulphuration pathway of the Hcy metabolism (Apeland et al., 2002).

In children, hyper-tHcy is defined according to age and (after puberty) sex-specific percentiles (Osganian et al., 1999). In a recent study, epileptic children with hyper-tHcy had significantly lower folate and cobalamin concentrations (Huemer et al., 2005). Moreover, multidrug AED treatment, age and duration of therapy correlated significantly with elevated tHcy and low folate levels. Folic acid supplementation alone, 1 mg/day for 3 months, resulted in a significant increase of folate and decrease of tHcy levels (Huemer et al., 2005).

An additional aspect to be considered is that women with low serum and red blood cell levels of folic acid are associated with increased incidence of spontaneous abortions and malformations (Ogawa et al., 1991; Kaneko et al., 1988). Noteworthy, maternal folate deficiency has been linked with the development of neural tube defects and periconceptional folate supplementation with a reduction of risk (Czeizel, 2004; Wolff et al., 2009). Although this has not been firmly established in women with epilepsy (Morrow et al., 2009), preconceptional and gestational folate supplementation at a daily dose of 0.4 mg has been recommended also in this population (Harden et al., 2009).

Interestingly, folinic acid has several advantages over folic acid which might, under some circumstances, offer a therapeutic advantage. It bypasses several steps in the conversion of folic acid to 5-MTHF; is more readily transported into the CNS than folic acid; has a longer half-life in the body; and it appears to be a more metabolically active form of folate, capable of boosting levels of the coenzyme forms of the vitamin in circumstances where folic acid has little or no effect (Kelly, 1998). Recently, the effect of 15 mg oral folinic acid on the blood tHcy level of hemodialysis patients has been compared with 15 mg folic acid. In this study, Soleimani et al. found that both folic and folinic acid decreased the tHcy serum level and no meaningful difference was observed between them, suggesting that they may be used interchangeably (Soleimani et al., 2011).

Conclusions

A number of studies suggest that lowering tHcy levels with folate and other B vitamins does not provide significant benefit in preventing cardiovascular events (Bønaa et al., 2006; Lonn et al., 2006; Holmes et al., 2011). However, the results of a recent meta-analysis provide the perception that many of the folate supplementation trials for stroke prevention might have failed because they were not undertaken in a low folate setting (i.e., countries with low folate status) (Holmes et al., 2011). An alternative view to interpret elevated plasma tHcy levels is that these are an indicator of folate and/or other vitamin deficiency, a condition which itself may have different clinical implications in patients with epilepsy (Reynolds, 2006; Harden et al., 2009). In fact, folate deficiency induced by phenytoin or barbiturates is commonly associated with mental changes, especially depression, apathy, psychomotor retardation, megaloblastic anaemia and cognitive decline (Reynolds, 2006). Folic acid alone or folic acid combined with other vitamins (i.e. B2, B6, B12) have all been shown to reduce tHcy concentration in patients on chronic treatment with AEDs and normalization of tHcy usually occurs within four to six weeks after the initiation of B-vitamins supplementation. It seems that there are small differences between high and low doses of folic acid as regards the effect on tHcy concentrations (Mansoor et al., 1999). However, which is the most appropriate supplementation of folic acid and/or B-vitamins in patients with epilepsy still remains matter of debate. Some literature data must keeping in the mind when prescribing B-vitamins supplementation trials in this population of patients: (i) long-term reductions in blood tHcy levels with folic acid and vitamin B12 did not have beneficial effects on vascular outcomes but were also not associated with adverse effects on cancer incidence (SEARCH Collaborative Group, 2010); (ii) folic acid can lower blood phenytoin concentrations and, in turn, aggravating epileptic seizures (Reynolds, 2006); (iii) chronic folate therapy may complicate the diagnosis of pernicious anemia (Reynolds, 2006). On the other hand, favorable clinical effects have been reported with B-vitamins supplementation and folic acid supplementation seem prevents phenytoin-induced gingival overgrowth in children (Arya et al., 2011). Moreover, folic acid 0.8 mg supplementation for 3 years significantly improved domains of cognitive function that tend to decline con age in older adults (Durga et al., 2007). Noteworthy, the administration of folic acid in the presence of vitamin-B12 deficiency (the so-called 'methyl-folate trap') may be harmful to the nervous system, after brief temporary improvement, and ultimately harmful to the blood, after more striking but suboptimal temporary improvement (Reynolds, 2006).

In conclusion, the upper limit of continuous folate intake that is commonly accepted to be safe is around 1 mg/day (Institute of Medicine, 1998), 0.4 mg of folic acid per day appears to be sufficient for patients on AEDs to restore tHcy into normal serum levels (Apeland et al., 2002; Harden et al., 2009; Bochyńska et al., 2012), continuous supplementation of high doses of folic acid may be harmful in individuals at higher risk for cancer (Sauer et al., 2009). Of note is that the benefits of vitamins B12 and B6 alone on tHcy are modest (Nilsson et al., 2001). For these reasons, we consider supplementation of 0.4 mg folic acid plus low B-vitamins doses (i.e. B2, B6, B12) as an option to treat or avoid consequences of folate deficiency in patients on chronic treatment with older AEDs.

Conflict of interest statement

V.B. has received travel and research grants from UCB Pharma and Pharmaline

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