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High Plasma Homocysteine and Low Serum Folate Levels Induced by Antiepileptic Drugs in Down Syndrome

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Abstract

Clinical and epidemiological studies suggested an association between hyper-homocysteinemia (Hyper-Hcy) and cerebrovascular disease. Experimental studies showed potential proconvulsant activity of Hcy, with several drugs commonly used to treat patients affected by neurological disorders also able to modify plasma Hcy levels. We assessed the effect of long-term AED treatment on plasma Hcy levels in patients with Down syndrome (DS) and epilepsy. We also evaluated the relationship between the plasma Hcy levels, and folic acid or vitamin B12. We enrolled 15 patients in the Down syndrome with epilepsy group (DSEp, 12 men and 3 women, mean age 22 ± 12.5 years old) and 15 patients in the Down syndrome without epilepsy group (DSControls, 12 men and 3 women, mean age 20 ± 13.7 years old). In the DSEp group the most common form of epilepsy was simple partial epilepsy, while the most common AED used was valproic acid. Plasma Hcy levels were significantly higher ($P < 0.01$) in the DSEp group compared with the DSControl group. Significant differences ($P < 0.01$) between DSEp and DSControls were also observed in serum concentrations of folic acid, but not in serum levels of vitamin B12. In conclusion, our observations suggest that treatment with AEDs in DSEp patients induces an increase in plasma Hcy levels and a significant decrease in serum folic acid, therefore supplementation with vitamins may be useful in order to obtain normal plasma Hcy values and reduce the risk of both cardiovascular and neurological diseases

Homocysteine (Hcy) is a thiol-containing amino acid that is formed by the demethylation of methionine, an essential amino acid derived from the animal proteins introduced with the diet (Selhub, Jacques, Wilson, Rush, & Rosenberg, 1993; Siniscalchi et al., 2005). It is metabolized through two major pathways: methylation and trans-sulfuration that are dependent on several cofactors. In particular, the methylation of Hcy to methionine requires folate and vitamin B12, whilst the trans-sulfuration to cysteine requires vitamin B6 (Siniscalchi, 2004; Siniscalchi et al., 2005; Siniscalchi, Gallelli, Mercuri, Fererri Ibbadu, & De Sarro, 2006).

The recycling of folate cofactors is dependent on vitamin B6 and B2; vitamin B2 is necessary to activate vitamin B6 to pyridoxal 5-phosphate (PLP) (Siniscalchi, 2004; Siniscalchi et al., 2005; Siniscalchi et al., 2006).

Clinical and epidemiological studies suggested an association between hyper-homocysteinemia (Hyper-Hcy), occlusive arterial vascular diseases (D'Angelo and Selhub, 1997; Perri, 1999; Refsum, Ueland, Nygard, & Vollset, 1998; Siniscalchi, 2004) and venous thromboembolism (den Heijer et al., 1996; 1998).

Hyper-Hcy represents both a reaction to acute illness and an important risk factor for cardiovascular mortality in patients with a history of myocardial infarction, stroke, angina pectoris, diabetes, or hypertension (Boysen, Brander, Christensen, Gideon, & Truelsen, 2003; Vollset et al., 2001).

Experimental studies have shown a potential proconvulsant activity of Hcy (Siniscalchi 2004) with several drugs commonly used to treat patients affected by neurological disorders also able to modify plasma Hcy levels. Clinical studies have reported that patients treated with old and new AEDs showed increased plasma Hcy levels (Belcastro et al., 2010; Linnebank et al., 2011; Schwaninger et al. 1999; Siniscalchi et al., 2005)

Moreover, folate deficiency occurs in some epileptic patients treated with AEDs such as valproate (VA) and carbamazepine (CBZ), and this effect may induce Hyper-Hcy (Kishi, Fujita, Eguchi, & Ueda, 1997). Therefore, vitamin B plays a role in altering plasma Hcy levels during AEDs treatment (Siniscalchi 2004; Siniscalchi et al.,2005)

In this paper, we assessed the effect of long-term AEDs treatment on plasma Hcy levels patients with Down syndrome and epilepsy. We also evaluated the relationship between the plasma Hcy levels, and serum levels of folic acid or vitamin B12.

Patients and Methods

Study Design

We undertook an open label, control-group, single centre study on patients with Down syndrome and seizures treated with AEDs and enrolled at the Cosenza Hospital. This study was approved by the Researchers' Ethics Committee and was conducted in accordance with the Declaration of Helsinki and the Guideline for Good Clinical Practice; all parents of participants provided written informed consent.

Patients eligible for the study were male or female, aged from 18 years up to 35 years, with primary epilepsy and prescribed AEDs (DSEp group).

Patients with the following conditions were excluded: allergy to AEDs, progressive serious medical conditions (i.e., cancer or AIDS), renal diseases (serum creatinine concentration more than 1.2 times the upper limit of the normal range according to the central laboratory definition reference values), liver dysfunctions (serum alanine transaminase or aspartate transaminase concentrations more than 1.5 times the upper limit of the normal range according to the central laboratory definition reference values) and drugs known to increase the plasma Hcy levels. Patients with Down syndrome and without epilepsy were also enrolled in our study and represented our control-group (DSControl group).

End Points

The primary end point was the effects of AEDs on plasma Hcy levels. Moreover, co-endpoints included the relationship between both the plasma Hcy levels and the vitamin B12 or folic acid levels.

Evaluation of Hcy, Vitamin B12 and Folic Acid Levels

For plasma Hcy levels evaluation, blood samples collected in heparin were immediately stored in fresh ice (T = 0°C) and then centrifuged (T = 18°C, 1500 g/min for 10 min). Plasma was take through a polyurethane pipette, put in a polyurethane tube and analyzed with high-performance liquid chromatography (HPLC-UV) in agreement with literature data (Amarnath, Amarnath, Amarnath, Valentine, & Valentine, 2003; Kuo, Still, Cale, & McDowell, 1997).

Serum levels of folic acid and vitamin B12 were analyzed by chemiluminescence with the AxSYM instrument in agreement with literature data (Bamonti et al., 2010).

Statistical Analysis

Statistical analysis was performed using SPSS. We evaluated the effects of drugs on the plasma concentration of Hcy, vitamin B12 and folic acid, but it is not easy to define a clinically significant change and therefore it is not possible to obtain a power calculation. Consequently, these results should be considered exploratory. The Student's *t* test was used to evaluate the change between groups. For all comparisons, differences were considered significant at $p < 0.05$.

Results

During the study we enrolled 15 patients in the DSEp-group (12 men and 3 women, mean age 22 ± 12.5 years old) and 15 patients in the DSControl-group (12 men and 3 women, mean age 20 ± 13.7 years old).

In the DSEp-group the most common form of epilepsy was simple partial epilepsy (Table 1), and the most common AED used was valproic acid, with a mean duration of treatment of 9.7 ± 3.0 years.

Plasma Hcy levels were significantly higher ($p < 0.01$), in the DSEp group compared with the DSControl group (Table 3). Significant differences ($p < 0.01$) between the DSEp group and DSC group were also observed in the serum concentrations of folic acid, but not in serum levels of vitamin B12.

Discussion

In this study we evaluated the effects of AEDs on plasma Hcy levels and serum vitamine B12 and folic acid levels in patients with Down syndrome (DS) with and without epilepsy.

Table 1. Epilepsy Manifestations in DSEp-Group

Simply Partial Epilepsy	*Generalized Epilepsy*	*Complex Partial Epilepsy*
7	5	3

Table 2. Antiepileptic Treatment of Patients with Down Syndrome and Epilepsy (DSEp-Group)

	Valproic acid	*Carbamazepine*	*Phenobarbital*
Treatment	5 mono-therapy 2 poly-therapy	4 mono-therapy 1 poly-therapy	3 mono-therapy
Mean time of treatment (years)	9.7 ± 3.0	10.1 ± 2.5	17.5 ± 3.2

Table 3. Concentrations of Homocysteine in Plasma, and Vitamin B12 and Folate in Serum. Values are Expressed as Mean ± Standard Deviation.

	DSControl Group	*DSEp Group*	*P Value*
Homocysteine (normal values 5–12 µmol/L)	8.2+2.0	14.8 ± 1.8	0.000
Vitamin B12 (normal values 145–914 pg/mL)	359.1 ± 35.1	354.6 ± 40.1	0.746
Folic acid (normal values 3–20 ng/mL)	17.9 ± 1.0	9.3 ± 1.5	0.000

Our data demonstrate that patients in DSControl-group show normal levels of folate and Hcy, while long-term treatment with AEDs in patients with DSEp may increase the plasma Hcy levels and this effect is related to a decrease in serum levels of folic acid. These data are in agreement with previous studies which demonstrated that patients with epilepsy treated with AEDs showed low serum folate and elevated plasma homocysteine (Belcastro et al., 2010; Linnebank et al., 2011; Schwaninger et al., 1999; Siniscalchi et al., 2005).

Although the mechanisms by which AEDs induce a decrease in serum folate levels are unclear, it has been hypothesized that AEDs may induce a decrease in intestinal absorption of folate, or an induction of liver enzymes (Siniscalchi et al., 2005).

The conclusions from previous studies on plasma levels of vitamin B12 in patients with epilepsy are controversial. Researchers have variously described not only a decrease (Refsum et al., 1998), as well an increase in concentrations of vitamin B12 (den Heijer, Blom, Gerrits, Rosendaal, & Haak, 1995), but also reported normal levels of vitamin B12 (Van Beynum, Smeitink, den Heijer, te Poele Pothoff, & Blom, 1999).

Recently, Linnebank et al., (2012) in a prospective monocentre study documented that the mean plasma levels of vitamin B6 were not significantly different between AED-treated, the untreated patients and the controls.

Thus further studies are needed to better analyze the relationship between AEDs and vitamins.

Plasma Hcy levels represent both an atherogenic and a thrombogenic risk factor in the development of cerebrovascular manifestations.

This could be important in patients with DS who have been documented to be more susceptible at the development of cardiovascular (Irving and Chaudari, 2012; Raina, McGrath, & Gunn, 2011) and neurological diseases (Lott & Dierssen 2010).

In patients with epilepsy, chronic treatment with AEDs induced an increase in plasma Hcy levels that represents a risk factor for the development of cerebrovascular diseases (Karabiber et al., 2003).

Patients with epilepsy receiving long-term AED therapy showed an alteration in circulatory markers of vascular risk that may contribute to a rapid increase of the atherosclerotic process (Chuang et al., 2012). With regard to a potential proconvulsant activity of Hcy, it has been documented that up to 20% of patients with homozygous cystathionine ß-synthase deficiency manifested seizures, therefore an increase in plasma Hcy levels (usually 50–200 μmol/L) may be able to induce epilepsy (Mudd et al., 1985).

In agreement, recent investigations in experimental animal models, showed that systemic administration of higher doses of Hcy are able to induce seizures (Marangos et al., 1990; Mares, Folbergrova, Langmeier, Haugvicova, & Kubova, 1997). However, at the moment it has not yet been demonstrated in humans whether lower levels of Hyper-Hcy (15–20 μmol/L), are able to induce epilepsy. Different mechanisms have been proposed to explain the convulsing properties of Hcy. The L-isomer of Hcy and its oxidized product, homocysteic acid, acts as an agonist at the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor sites (Lipton et al., 1997; Schwarz, Zhou, Katki, & Rodbard, 1990) and at metabotropic glutamate receptors (Shi et al., 2003). Another hypothesis suggests that Hcy could increase the adenosine uptake able to reduce the seizure threshold (McIlwain & Poll 1985). Thus Hcy may also increase seizure frequency in patients treated with AEDs. Therefore, the decrease of plasma Hcy levels could lead to a decrease of seizure activity.

Clinical studies (Apeland et al., 2002; Siniscalchi et al., 2005) reported that the supplementation with folate, vitamin B12 and B6 in patients with Hyper-Hcy induced by AEDs decreasesd the plasma Hcy levels and influenced endothelial activation, showing that the B group of vitamins have a vascular-protective effect in patients administered AEDs.

Previously it has been suggested that in men and women treated with AEDs, supplementation with folic acid tablets (1 mg each) or as part of a multivitamin supplement could be useful in order to normalize the Hcy plasma levels (Morrell et al., 2002).

Fillon-Emeryet et al. (2004) showed in adults with trisomy 21 that Hcy levels were decreased

by folic acid administration. Therefore, supplementation with this vitamin may also be able to reduce plasma Hcy levels in patients with DS and epilepsy treated with AEDs.

However, based on our data showing that DS patients without epilepsy don't show an increase in Hcy plasma levels or a decrease in folate levels, we hypothesized that folic acid may be used in patients with DS and epilepsy treated with AEDs even if a deficit of folate has not been reported.

In conclusion, our observations suggest that treatment with AEDs in patients with DS and epilepsy induces an increase in plasma Hcy levels and a significant decrease in serum folic acid. Therefore supplementation with this vitamin may be useful for achieving normal plasma Hcy values and consequently reduce the potential for the development of both cardiovascular and neurological.

Key Messages From This Article

- Patients with DS show normal levels of Hcy and folate.
- AEDs treatment induced in patients with DS and seizures an increase in Hcy plasma levels with a reduction in folate serum levels.
- The increased levels of Hcy could represent a risk factor for cardiovascular and neurological diseases in patients usually more susceptible at these manifestations.
- The addition of folate through diet or vitamin supplements could normalize the levels of Hcy.

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