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# Homocysteine, vitamin determinants and neurological diseases

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## 1. ABSTRACT

This review focuses on the putative role of hyper-homocysteinemia in the pathogenesis of different diseases affecting the nervous system, including stroke, Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis and amyotrophic lateral sclerosis. However, a firm pathogenic role of homocysteine in these diseases has never been established. Lowering plasma homocysteine levels trough vitamin therapy failed to prevent vascular diseases. Conversely, normalization of hyper-homocysteinemia caused improvement in patients with cognitive impairment. B vitamin deficiency is the main determinant of homocysteine levels. However, it has been hypothesized that homocysteine might be a mere marker of vitamin deficiency or an indicator of disease rather than a risk factor. A more consistent use of thresholds to define deficiency is needed to recommend routine screening, monitoring and supplementation of B vitamins to ameliorate the prognosis of the above mentioned disorders. To date, data are insufficient to firmly establish which one of the hypotheses made is correct and the question concerning the real meaning of hyper-homocysteinemia in the pathology of the nervous system still remains an intriguing medical dilemma.

## 2. INTRODUCTION

Homocysteine (Hcy) is a sulphur-containing amino acid produced by de-methylation of the dietary essential amino acid methionine (1). In physiological conditions plasma total (t) Hcy levels are <15 μmol/L, as reported by the majority of investigations. Less frequently, a threshold of 13 μmol/L has been reported, this depending on the method used (2). The medical interest in this amino acid started in 1969, when a report highlighted that elevated urinary concentrations of Hcy (homocystinuria) in children with inborn errors of Hcy metabolism were associated to vascular damage (3). Under this condition, plasma tHcy concentrations may rise up to or even above 200 μmol/L (a condition defined as "severe" hyper-Hcy) and displays a clear thrombotic effects (4, 5). Since then, an abundant worldwide clinical research has been carried out to establish the possible causative role of supra-physiological plasma tHcy levels in the development of vascular and neurodegenerative diseases, including coronary hearth disease, stroke, and brain atrophy (6-11). A number of factors, such as age, plasma folate and vitamin B12 concentrations, serum creatinine, alcohol consumption, dietary restrictions and different pathological conditions, such as diabetes, hypertension, renal insufficiency and

others, can be associated to elevated plasma tHcy levels (2). Experimental data strongly support that Hcy may contribute to the progression of atherosclerosis via endothelial dysfunction through oxidative stress (12). This effect could be mediated by formation of reactive oxygen species (ROS) (13), including superoxide anion and hydrogen peroxide, increased lipid peroxidation (14), induced nitrosative stress (15) and impaired production of the antioxidant glutathione peroxidase (16). Additionally, in the nervous tissue Hcy and its derivatives, such as homocysteic acid, function as excitatory amino acids by activating N-methyl-D-aspartate (NMDA) receptor subtype (17). It has been observed in cerebellar neurons that Hcy-induced activation of NMDA receptors elicits only excitation, while normal activation can induce both excitation and inhibition (18). Thus, Hcy might act through excitotoxic mechanisms.

In a clinical setting, the hypothesis that hyper-Hcy plays a causative role in neurodegenerative and cerebrovascular diseases is very attractive since it is an easily modifiable factor and hence a preventive therapy could be promptly implemented. However, recent large collaborative trials have concluded that lowering plasma tHcy with folic acid and/or other B vitamins does not result in any clinical benefit in the prevention of stroke and hearth coronary diseases (19, 20). Some criticism has been raised up especially on the size of the populations studied in these studies, not so large to confer a sufficient power to the statistical analysis (21). To explain the whole of the conflicting data of the literature, in recent years a line of interpretation is growing, which regards hyper-Hcy as a bio-marker rather than a causative factor (22). Thus, the scientific interest is recently expanding on the possible effects displayed by folate and other B vitamins when brain cells are exposed to chronic oxidative insults (23). Folic acid is an important cofactor in the Hcy methylation, the primary means of regulating Hcy concentration (6). Usually, a condition of elevated plasma tHcy levels is paralleled by low folate levels and folate supplementation results in normalization of Hcy concentrations (24, 25). Apart from this effect, a good intake of folic acid appears to offer some protection against tissue damage through different mechanisms (26). Data of the literature have suggested that folate and other B vitamins modulate redox-dependent mechanisms leading to up-regulation of brain response and hence potentiate brain tolerance to oxidative stress (23). On these basis, new neuroprotective strategies are developing, especially those aimed at minimizing deleterious consequences associated to oxidative stress (27, 28).

In this chapter we will review the most recent findings concerning the biochemical changes caused by supra-physiological plasma tHcy concentrations in the central nervous system (CNS) and discuss the possible role played by folate and other B vitamin deficiency in supporting an association between hyper-tHcy and neurodegenerative disorders.

## 3. HOMOCYSTEINE METABOLISM

Multiple enzymes and different cofactors are involved in Hcy metabolism. 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 (29). 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. The activated S-adenosyl methionine (SAM) is required for several trans-methylation reactions involving different substrates such as phospholipids, myelin, choline and cathecolamines. S-adenosyl-methionine (SAM) generated in these reactions is in 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 (Figure 1).

Hcy in plasma exists in different forms, including trace amounts of reduced Hcy (1-2%), free oxidized fraction (10-20%) where cysteine-Hcy mixed disulphide predominates, and the major protein-bound fraction (80%).

## 4. GENETIC POLYMORPHISMS INVOLVED IN HCY 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 substitution 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* (30, 31). MTHFR gene C677T and A1298C polymorphisms are common in the general population (32, 33). The T677 allele occurs in 35% of Caucasian populations and TT homozygotes may achieve a percentage of 10-20% (34). Carriers of the TT677 MTHFR genotype frequently exhibit elevated plasma Hcy, especially if stores of folate or vitamin B12 are depleted (35). The TT mutation has been described to result in reduction of the MTHFR enzymatic activity >50% (32). The A1298C polymorphism alone does not affect appreciably plasma Hcy but it may do so when combined with the 677T variant (AC/CT combination) (36).

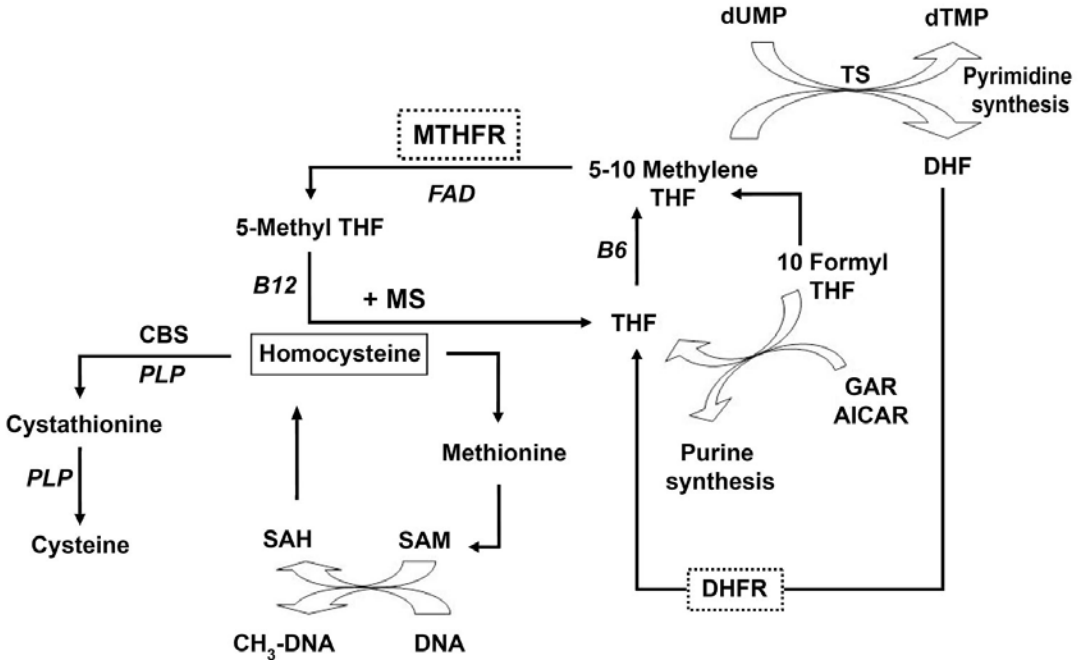


**Figure 1.** Hcy metabolism and involved vitamin determinants.Hcy is an intermediary in sulfur amino acid metabolism. The methionine cycle and the folate cycle are involved in Hcy formation via the numerous methyl transfer reactions. The metabolism of Hcy is also dependent on several vitamins, including folate, B12 (cobalamin), B6 (pyridoxine).AICAR, aminoimidazolecarboxamide ribotide; CBS, cystathionine beta-synthase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; FAD, flavin adenine dinucleotide; GAR, glycinamide ribotide; MS, methionine synthase; MTHFR, methylene tetrahydrofolate reductase; PLP, pyridoxal phosphate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; TS, thymidylate synthase.

With regard to the A2756G methionine synthase (MS) polymorphism, functional data are limited and inconsistent (37).

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 (38). 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 (39). Patients with homozygous homocystinuria have a severe deficiency of CBS activity and exhibit plasma tHcy levels very high (> 100 μmol/l). The excess of Hcy is excreted into the urine as homocystine. About 1% of the population has mutations in the gene of the CBS in heterozygous form (40).

Other mutations exerting a possible influence on Hcy metabolism have been described (41, 42). However, these disorders are rare and their clinical relevance has not been explored to date.

## 5. HCY-MEDIATED MECHANISMS OF TOXICITY IN THE NERVOUS SYSTEM

### 5.1. Excitotoxic effects

The over-stimulation of glutamate receptors is today considered a common feature in the pathogenesis of several neurodegenerative conditions (43). Alterations in intracellular calcium homeostasis has been demonstrated to mediate the toxicity of glutamate and NMDA on neurons (44). Among excitatory aminoacids, Hcy is toxic to human and murine neuronal cells *in vitro* (45). Hcy showed binding activity toward NMDA subtype of glutamate receptors, acting as agonist and partial antagonist at glutamate and glycine binding site, respectively (46). It has been reported that biochemical events associated to excitotoxicity, such as increased calcium levels in the cytosol, increased generation of reactive oxygen species and apoptosis, are important hallmarks of several neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's Disease (PD) (47, 48).

### 5.2. Oxidative effects

It has been reported that Hcy hinders DNA repair in hippocampal neurons and sensitizes them to oxidative stress (49). In differentiated human neuroblastoma cell models, Hcy also potentiates the beta-amyloid-induced increase in cytosolic calcium and apoptosis (50). Additionally, *in vitro* studies using cerebellar granule cells have demonstrated that Hcy-mediated cell death can be prevented by co-administration of superoxide dismutase (SOD) and catalase or by catalase alone (51). These observations suggest that formation of hydrogen peroxide contributes to Hcy-mediated cell death.

Plasma membrane carrier-mediated uptake is present in neurons and glial cells and this allows to

hypothesise that Hcy can be imported from plasma into the brain (52). tHcy concentrations in plasma have been described to be 20–100-fold higher than those assayed in the CSF (52-55).

Recent results indicate that one of the possible mechanisms of Hcy-induced neurotoxicity is inhibition of cytochrome oxidase activity by Hcy-mediated $Cu^{2+}$ chelation, finally leading to mitochondrial dysfunction (56). Additionally, high tHcy levels have been seen to cause an imbalance in the redox status homeostasis with generation of reactive nitrogen and oxygen species (RNS/ROS) in endothelial cells (57). The excess of ROS production, affecting cell cycle arrest and apoptosis, might be responsible for neuronal cell damage.

### 5.3. Inflammatory effects

Apart from the above illustrated oxidative and excitotoxic mechanisms, another interesting field of Hcy-mediated cell damage regards the relationship between Hcy and inflammatory processes. Folic acid and vitamin B12 deficiency have been independently associated with decreased immune function, the apoptosis of bone marrow hematopoietic progenitor cells and the appearance of leukocytes with hypomethylated DNA in the peripheral circulation (58). Moreover, gene mutations and gene silencing are known to play a critical role in the inactivation of genes involved in DNA repair, in cell cycle control, in proper chromosome segregation during mitosis, and in apoptotic pathways (59).

Several processes involved in the repair of DNA damage result in an increased requirement for methylation of DNA, RNA and proteins. These reactions lead to the generation of Hcy as the end product. Optimal concentrations of folates are essential in that they serve also as donors of 1-carbon units in the biosynthesis of the purine ring of DNA and in the production of methyl groups. In addition, there is evidence suggesting that oxidative stress resulting from immune activation may lead to the oxidation of folates, resulting in folate deficiency despite a normal dietary intake. Thus, hyper-Hcy may be a consequence, rather than the cause, of inflammation and oxidative degradation of folates. Plasma tHcy, as an end product of tissue repair, may therefore be an indicator of inflammatory disease processes (60).

Since the decline of T cell immune function during aging is well documented, it has been suggested that T cells might be an ideal system to study *in vitro* the potential role of replicative senescence during *in vivo* aging (61, 62). Interestingly, it has been reported that Hcy is able to promote T cell activation and differentiation in a concentration-dependent way, and to potentiate activation-induced cell death and apoptosis (63).

Function and longevity of immuno-competent T cells may be affected by signalling processes, such as agonist-dependent receptor activation and ROS production (64-66). In this context, it has been described in a model of immunosenescence, that Hcy evokes apoptotic effects (63, 67, 68).

Increase in Hcy levels in the cell nuclear compartment produces DNA strand breaks by disturbing the DNA methylation cycle (69). Although the underlying mechanisms promoting DNA damage have not yet been elucidated, it is possible to hypothesize an involvement of ROS production (45, 70). Some evidence suggests that different approaches may be useful to counteract the toxic effects of Hcy, including quenching of oxidative agents and maintenance of trans-methylation pathways (71).

Hcy-evoked effects may further trigger cell damage in the circulating cell compartment, potentially leading to modulatory effects on immune function. Indeed, elevated plasma tHcy concentrations are associated to a number of inflammatory diseases and immune-cell activation states such as psoriasis (72), systemic lupus erythematosus (73), rheumatoid arthritis (74), malignancies (75), and immune activation in Parkinson's disease (76). Therefore, the identification of molecular mechanisms associated with Hcy-induced cell cycle dysregulation may permit specific targeting of cell cycle in the prevention of Hcy toxicity in various age-related pathological conditions.

A growing body of evidence suggests that ROS-induced cell damage results from or is accompanied by inflammatory processes, in which the activation of NF-kappaB plays a pivotal role. Recently, we have observed that Hcy exposure increases NF-kappaB levels, while antioxidants, particularly IRFI 016 (80 μM), are able to counteract NF-kappaB activation induced by 250 μM Hcy (77). Additionally, in the same experiments cell treatment with 250 μM Hcy triggered the onset of apoptosis, as demonstrated by the increased expression of early apoptotic markers such as Bax, caspase-3 and p53. In contrast, Bcl2 expression, an anti-apoptotic factor, was not affected by Hcy exposure (77). These results suggest that increase in NF-kappaB DNA binding activity may be associated to cell damage in apoptotic cells.

A number of inflammatory genes, the products of which are putatively involved in apoptosis, are regulated by NF-kappaB (78, 79). However, the role of NF-kappaB in neuronal death is controversial, and the effects of NF-kappaB nuclear translocation in cell response still remain unclear.

## 6. CEREBROVASCULAR DISEASE

In the past decades no-modifiable risk markers for stroke, such as age, gender, race, ethnicity, heredity and several modifiable risk factors for ischemic stroke, such as smoke, hyper-glycaemia, hyper-cholesterolemia, etc., have been identified (80-82).

Several epidemiological observations have linked hyper-tHcy to increased risk for stroke (83-85). One of the possible mechanisms involved may relate to induction of endothelial dysfunction and/or chronic inflammation, as above illustrated. Interestingly, hyper-tHcy has been associated with a decreased bioavailability of nitric oxide through generation of asymmetric dimethylarginine (ADMA), an analogue of L-arginine,

which is a competitive inhibitor of eNOS (86). ADMA also increases the production of superoxide and other ROS, which reduce NO availability. Production of ADMA results from an increase in the methylation of proteins and has been associated to Hcy rise (87). Inhibition of endothelial nitric oxide synthesis by ADMA impairs cerebral blood flow, which may contribute to the endothelial dysfunction and cognitive impairment (88).

A number of observations has suggested that mild (i.e. plasma tHcy levels not higher than 30 μmol/L) hyper-Hcy may be a risk factor for cervical artery dissection causing ischemic stroke, as confirmed by a recent study showing an association of hyper-Hcy with ischemic stroke due to atherothrombotic or small artery disease (84). On this basis, the possibility that treatments lowering plasma Hcy, namely vitamin supplementation, has been explored. Unfortunately, large intervention studies produced conflicting data, some showing benefits against stroke (89, 90) and some others no effects against cardiovascular events (20, 91). However, some criticism has been advanced, especially on the size of the populations studied, not sufficiently large to confer a right power to the statistical analysis (90). Similarly, conflicting results have been produced by studies aimed at investigating the effect of Hcy lowering by folate intake on endothelial dysfunction thought to be caused by supra-physiological plasma tHcy levels (92).

It may be hypothesised that the time of B vitamin intervention in relation to the stage of the disease plays a crucial role in producing beneficial effects and might explain the conflicting findings described.

## 7. COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

Several reports have hypothesised that abnormally high plasma tHcy levels, as frequently occurring in elderly people, might be associated to cognitive decline and dementia (93).

In AD high concentration of copper have been found near to beta-amyloid deposits suggesting a production of reactive oxygen species (94). Hcy also increases the levels of calcium and causes production of reactive oxygen species (ROS) in presence of transition metals (95). In line with these results, a recent study supports the view that high tHcy levels may increase the risk of AD (96, 97).

In AD patients, the impairment of methylation pathway, resulting from elevated levels of Hcy and low levels of S-adenosylmethionine (SAM), may be associated to alterations of DNA methylation (98). It has been reported that addition of SAM to cell lines down-regulates the expression of different genes involved in beta-amyloid formation (99). Similar results were obtained in different studies by addition of folate and vitamin B12 which are able to influence DNA methylation (99, 100). In addition, the auto-oxidation of Hcy is known to generate ROS, whereby the prevention of Hcy-induced toxicity by catalase suggests that hydrogen peroxide acts as a mediator of oxidative injury, leading to apoptosis (101, 102). Moreover Hcy inhibits both the expression of antioxidant enzymes and the synthesis of radical scavengers (e.g. glutathionine peroxidase, superoxide dismutase) which might potentiate the toxic effects of ROS (103).

In one of our investigations, exposure to Hcy in presence of antioxidants, such as N-acetylcysteine (500 μM) and IRFI 016 (80 μM), a synthetic alpha-tocopherol analogue, recovered cell viability and significantly counteracted Hcy effects, most likely through restoration of ROS basal levels (77).

It may be also relevant that high levels of tHcy dependent on folate deficiency are implicated in DNA damage in the CNS (104). In this regard, as stated above, impaired DNA methylation and associated mechanisms may increase beta-amyloid production and toxicity (69). Some data have shown that folic acid deficiency and elevated Hcy levels may contribute to increase the vulnerability of cultured hippocampal neurons to beta-amyloid-induced death and to promote neuronal degeneration (49). Both oxidative stress and DNA damage have been documented in neurons associated with beta-amyloid-containing plaques in the brains of AD patients (for review, see 105). Thus DNA damage and repair suggest that folic acid deficiency and hyper-tHcy promote the accumulation of DNA damage in neurons by impairing DNA repair.

In this context, postmitotic cells have been shown to be more vulnerable to DNA damage than mitotic cells, probably because of the lack of efficacy of DNA repair (49). In fact, it was shown that neurons and neuroblastoma cells become extremely UV-sensitive after terminal differentiation(49). Therefore, in mature neurons the mechanisms involved in the cell cycle activation can be an important component of the mechanisms associated to DNA damage leading to cell death. The exposure of cultured neurons to beta-amyloid induces caspase activation (106) and increased production of Par-4 and Bax, each of which appears to play an important role in the cell death process (107). Apoptosis triggered by DNA damage typically involves activation of PARP and induction and activation of the tumor suppressor protein, p53 (108). Increased PARP activity and p53 levels have been documented in association with degenerating neurons in AD patients and in cultured neurons exposed to beta-amyloid. Moreover, a chemical inhibitor of p53 can protect neurons against beta-amyloid toxicity, suggesting a key role for this pathway, involving DNA damage-responsive cell death, in the pathogenesis of AD (109).

These and other findings suggest that folic acid deficiency and elevated Hcy levels, by impairing the DNA repair capacity in neurons, reduce the threshold level of DNA damage that is required to trigger neuronal death (110) and accelerate the accumulation of DNA damage that is promoted by age-related increases in oxidative stress and by accumulation of beta-amyloid.

Moreover, neurons are more vulnerable to DNA damage and it is well recognized that adequate availability of essential nutrients involved in cellular one-carbon metabolism is essential for normal brain development and function. The alterations in methyl group metabolism, associated to folate deficiency, leads to an imbalance in cellular antioxidant defense systems, increased oxidative stress, and cell damage. Any of these events may compromise normal CNS function and contribute to the development of various neurological, and neurocognitive dysfunctions (111).

In humans the normal range of plasma tHcy concentrations is 5–15 μM (2) and Hcy levels in CSF and brain tissue have been reported to range from 0.5 to 10 μM (112). Plasma folic acid levels decrease and Hcy levels increase with age and, even to a greater extent, in patients with AD and PD (96, 113, 114). In patients with AD at an advanced stage or with stroke a population of neurons degenerate and axons will not be replaced; in this case, vitamin supplementation did not improve brain function (115).

## 8. PARKINSON'S DISEASE

A number of studies has demonstrated that patients with Parkinson's disease (PD) exhibit plasma Hcy levels above the physiological range. These findings may be explained by chronic intake of levodopa (L-DOPA) (116-118). This drug, in fact, is metabolized via O-methylation by catechol-O-methyl-transferase (COMT) using S-adenosyl-L-methionine (SAM), the main methyl group donor in the brain, as substrate. L-DOPA, depleting the methyl groups required for Hcy conversion to methionine, may ultimately lead to hyper-tHcy. We have investigated the effects of C677T MTHFR polymorphism, in association with vitamin status and LD treatment, on hyper-tHcy development in a population of PD patients (119). In agreement with other authors (120, 121), a mild hyper-tHcy was observed in our population, plasma tHcy levels ranging from 11.2 ± 1.6 μmol/l in individuals with the CC + AA genotype to 22.1 ± 4.9 μmol/l in those with the TT + AA genotype. In our study, a strong positive correlation between Hcy levels and L-DOPA daily dose was observed (119). Previous data, however, have shown that hyper-Hcy in PD patients is more strongly associated with the duration of PD and L-DOPA treatment than with L-DOPA doses (120).

Theoretically, lowering plasma tHcy levels in PD patients might be important in view of the possible clinical implications. There is strong evidence, in fact, that Hcy displays detrimental effects on the dopaminergic system through excitotoxic mechanisms (122). Hyper-tHcy, therefore, might play a role in speeding up disease progression.

## 9. EPILEPSY

Epilepsy is a common neurological disorder which is phenotypically and aetiologically heterogeneous. Epileptic patients exhibit, in a percentage of 20-40%, supra-physiological plasma levels of tHcy. This is a consequence of the interplay between variants of the MTHFR gene and the chronic intake of antiepileptic drugs (AEDs), which deplete the organism of folates through induction of the hepatic enzymes (123).

In a previous study we have observed a more frequent association between MTHFR gene 677C>T and A1298C polymorphisms in epileptic patients than in the control sample (124). This is not a completely new finding and, indeed, some previous observations have suggested an association between the common 677C>T polymorphism in the MTHFR gene and epilepsy (125, 126). Carriers of AC/CT combination and concomitant low folate concentrations exhibited the highest tHcy plasma levels (124). Thus, both C677T and A1298C MTHFR polymorphisms should be examined when assessing genetic risk factors of Hcy in patients with epilepsy (124). In this population, hyper-tHcy has been suggested to contribute to the development of atherosclerosis (127), brain atrophy (11), and possible recurrence of seizures, as suggested by experimental data in animals (128). Recently, pulses of 5mg/day folate supplementation over the year have been suggested to normalize plasma levels of both folate and tHcy in epileptic patients with MTHFR polymorphisms (129).

## 10. MULTIPLE SCLEROSIS

Hyper-tHcy has been also observed in a certain percentage of patients with multiple sclerosis (130). Alterations of blood brain barrier and Hcy over-production deriving from activated astrocytes could be responsible for this finding (131).

Recently, evidence has been produced supporting a link between hyper-tHcy and cognitive impairment in patients with multiple sclerosis (132).

In some circumstances it may be suggested that vitamin B12 deficiency, associated to high levels of tHcy, may sensitize patients to the immunological mechanisms of MS (133). Different studies have analyzed vitamin B12, folate, and Hcy levels in patients with MS and found that vitamin B12 deficiency may present with different symptoms (131, 133).
Considering that vitamin B12 is involved in myelin formation and immune function, its deficiency might be of relevance in the pathogenesis of MS.

## 11. MOVING TOWARD A DIFFERENT VIEW: HCY AS AN INDICATOR OF VITAMIN STATUS

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 (23, 134). For example, epidemiological studies have shown that maternal folate deficiency is associated with the development of neural tube defects and periconceptional folate supplementation with a reduction of risk (135, 136). Recent USA guidelines

have recommended a preconceptional and gestational folate supplementation at a daily dose of 0.4 mg in women with epilepsy. On the basis that the bioavailability of natural food folate is incomplete, folate fortification has been adopted in U.S.A. to minimize the problem (137). However, possible adverse effects of excessive folate supplementation should be also taken into consideration. For example, excessive folate intake might be harmful in individuals at higher risk for cancer and cardiovascular disease (138).

Concerning the mechanisms through which folate can display its effects, different actions have been elucidated. Kruman *et al.*(49), for example, has reported that dietary folic acid deficiency significantly reduces the number of proliferating cells in the dentate gyrus of the hippocampus in adult mice. Cerebral lesion volumes after middle cerebral artery (MCA) occlusion and reperfusion have been described to be larger in folic acid-deficient 129/SV wild-type mice than in normally fed controls (139). Additionally, in this study, the authors observed that abasic sites, hallmarks of oxidative DNA damage were significantly increased in DNA from the ischemic brains of folate-deficient 129/SV wild-type mice soon after MCA occlusion (139).

DNA synthesis requires methyl groups, which are, at least partly, produced by methionine. As described above, Hcy is re-methylated to methionine by enzymes that require folic acid or cobalamin (140, 141). Folic acid deficiency may facilitate development of different age-related diseases, including coronary artery disease (142, 143), stroke (144), and cancers (69). In line with these effects, a number of observations has suggested that folic acid significantly influences repair mechanisms in the adult and in the developing nervous system (145). In many regeneration models folates play a role in different functions in the brain including growth, differentiation, cognition and ageing (146).

## 12. CONCLUSIONS AND PERSPECTIVES

As discussed above, an abundant literature supports the view that supra-physiological levels of Hcy are a risk factor for cerebrovascular and neurodegenerative diseases. Epidemiological studies and clinical investigations, in fact, have associated hyper-Hcy to stroke, epilepsy, Parkinson's disease, Alzheimer's disease and multiple sclerosis. Recently, high Hcy levels have been observed in patients with amiotrophic lateral sclerosis (147).

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 peroxidase.

Despite of the whole of these data, an as much great number of investigations failed to demonstrate that Hcy plays a pathogenic role in the above listed neurological diseases and indeed the view of hyper-Hcy as a causative agent is long far from achieving a firm consolidation. Recent large intervention studies were unable to demonstrate that lowering Hcy levels through folate and other B vitamin supplementation results in a benefit for stroke, coronary hearth disease and sudden death from cardiovascular disease. Therefore, in more recent years the concept that hyper-Hcy may be an indicator rather than a causative agent of pathology has started to make way in the scientific community. Indeed, high plasma tHcy levels are a sensitive marker of folate and vitamin B12 deficiency and can be related to several gene mutations as well as epigenetic factors involved in the complex methionine pathway. In this sense, hyper-Hcy might be considered a bio-marker of a given pathologic process induced by other triggering factors, in which, for example, folate and/or vitamin B12 are involved. To conclude, the matter of hyper-Hcy as a cause or a marker of a given pathology still remains an intriguing, complex and fascinating medical dilemma requiring a long scientific way to be fully clarified.

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