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Review

Critical levels of brain atrophy associated with homocysteine and cognitive decline

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ABSTRACT

Few B-vitamin trials to lower homocysteine (Hcy) have reported evidence of beneficial effects on cognition in older adults with cognitive impairment or Alzheimer's disease. This article reviews the role of Hcy in cognitive decline. It also considers some reasons why meta-analyses have failed to find effects of B-vitamin treatment. Findings from the successful VITACOG trial are examined from a new perspective of critical levels of Hcy and brain atrophy that may impact on the efficacy of B-vitamin treatment. It appears that there is a critical level of brain shrinkage, possibly mediated by elevated Hcy, which when reached, results in cognitive decline, especially in episodic memory performance. Supplements, food sources, and effects of folic acid fortification are discussed in relation to B12 deficiency.

1. Introduction

Risk factors for Alzheimer's disease (AD) affecting the rate of cognitive decline and brain shrinkage include nonmodifiable factors such as age, low education levels, and genetic factors, whereas modifiable risk factors have also been identified. One such modifiable risk factor is homocysteine (Hcy), an amino acid that is produced in the methylation cycle of protein metabolism. The association between elevated plasma Hcy and cognitive impairment has been well established (Budge et al., 2002; McCaddon et al., 2001; Seshadri, 2006), although the underlying mechanisms to explain the association are still being researched.

1.1. The Hcy pathway

Hcy is produced via protein metabolism. The conversion of Hcy to useful metabolites, *S*-adenosyl methionine and glutathione, requires vitamins B9 (methyl folate), B12 (cobalamin), and B6 (pyridoxine) as cofactors (Morris, 2012a; Refsum et al., 2006). Hence, if the B-vitamin supply through the diet is suboptimal, remethylation of Hcy via the enzyme methionine synthase is reduced, and plasma levels of Hcy rise. The importance of the remethylation process is the regeneration of the active form of folate, tetrahydrofolate, needed for thymidine synthesis, DNA replication, and neurogenesis. *S*-adenosyl methionine is a methyl donor for the central nervous system and important to neurotransmitter synthesis. Vitamin B12 is also important for fatty acid metabolism, acting as a cofactor for the enzyme methylmalonyl-CoA mutase and also promoting neural membrane formation. Buildup of methylmalonic acid (MMA) indicates the loss of this B12 function. Disruption of any of these pathways is likely to lead to loss of cognitive function and contribute to neuronal atrophy. Increased oxidative stress occurs in the brain when Hcy is elevated (Birch et al., 2009) and may increase the permeability of blood brain barrier. It is well known that vitamin B12 deficiency is a cause of pernicious or megaloblastic anemia, peripheral neuropathy, lack of energy, and poor memory.

Hcy levels rise with age (Nygård et al., 1998), possibly because of poor absorption of B vitamins from the diet and other factors including male sex, smoking, high blood pressure, and other clinical conditions (Refsum et al., 2006). However, studies in AD patients

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showed that blood levels of total Hcy are higher than in healthy controls, whereas folate and B12 levels are lower (Clarke et al., 1998).

1.2. Brain shrinkage, Hcy, and cognitive decline

Brain shrinkage because of cortical atrophy occurs with normal aging (Fjell et al., 2009; Thambisetty et al., 2010). The rate of brain atrophy has been shown to be a marker of cognitive decline (Fox et al., 1999) in domains such as memory, processing speed, and executive function (Fjell and Walhovd, 2010).

A 5-year study of people older than 60 years showed that percentage brain volume loss occurred at an average rate of 0.7% ± 0.3% per year (Vogiatzoglou et al., 2008). However, the decrease in brain volume was greater among those with lower vitamin B12 levels and markers of functional B12 including holo-transcobalamin at baseline. Brain volume loss was also associated with higher plasma total Hcy and MMA levels at baseline. For those with the lowest tertile of baseline vitamin B12 (<308 pmol/L), there was a 6-fold increase in the rate of brain volume loss. Elevated total Hcy is also associated with a smaller hippocampus in community-dwelling older adults (Williams et al., 2002). Minimal hippocampal width was shown to decline by 0.7 mm for each 10 micromolar increment in Hcy.

The rate of brain atrophy is known to be increased with neurodegenerative diseases such as AD. Atrophy in the medial temporal lobes becomes detectable by brain imaging at an early stage and is followed by increasing atrophy spreading to other regions of the brain in a sequential pathway (Smith, 2002). Rates of atrophy in those with AD can reach up to 12% per year and have been directly associated with cognitive decline starting in the domain of episodic memory and later involving domains of attention, executive function, processing speed, language, visuospatial skills, and orientation.

Hcy levels have also been associated with cognitive decline. For example, the Hordaland Homocysteine Study found that a rise in Hcy levels over time (6 years of follow-up) predicted cognitive decline (Nurk et al., 2005). This association has been confirmed in other studies (McCaddon et al., 2001) and reviews (Sachdev, 2005) and shown to be age dependent (Oulhaj et al., 2010). Controversies to these findings have been discussed by Morris (2012a).

2. Interventions with B vitamins

Randomized controlled trials (RCTs) to delay cognitive decline with B vitamins have been performed in participants with and without cognitive impairment and AD. Most reviews of these RCTs have shown little support for the efficacy of interventions by meta-analysis of data (Clarke and Bennett, 2008; Malouf and Grimley Evans, 2008), even when selecting only studies >6 months long, those in countries without folic acid fortification, those that included folic acid, and those with >100 participants (Ford and Almeida, 2012). Longer intervention, larger sample size, and the suggested efficacy of folic acid supplementation should have improved the likelihood of showing treatment effects of B vitamins. However, there were other issues that may have limited the findings. These included analyses of studies with normal participants combined with those with hypertension, cardiovascular disease, low baseline B-vitamin status, or normal baseline Hcy status. They also included analyses with cognitively impaired participants combined with those with mild impairment and moderate AD and trials that used insensitive cognitive outcomes such as the mini-mental state examination. The few RCTs with positive results of B-vitamin treatment not only used selective inclusion criteria (e.g., high baseline Hcy, participants with mild cognitive impairment or mild AD), had large sample sizes and long-term interventions, but also used sensitive domain-specific cognitive outcome measures for episodic memory, processing speed, and executive function (de Jager et al., 2012; Durga et al., 2007), for example, and the VITACOG trial used subtraction magnetic resonance imaging for detecting rate of brain shrinkage as a marker of treatment efficacy (Smith et al., 2010).

The folate after coronary intervention trial (FACIT) showed improved memory and processing speed performance in normal older adults with elevated baseline Hcy after 3 years of intervention with high-dose folic acid (800 μg per day). However, the dose was not very high. The VITACOG Hcy-lowering trial showed a reduced rate of brain shrinkage per year of up to 53% with high-dose treatment with 3 B vitamins (B6, B9, and B12) for 2 years. The reduced rate of brain shrinkage was dependent on the baseline Hcy level, with the most reduction observed in those with Hcy >13 μmol/L (Smith et al., 2010). Furthermore, the reduction was shown to be in those areas most relevant to AD pathology by voxel-based morphometry measures of structures including the hippocampus, parahippocampal, fusiform, right angular, right supramarginal, lingual, and inferior temporal gyri, and precuneus (Douaud et al., 2013).

The study also showed efficacy for cognition in those who started the study with Hcy levels >11.3 μmol/L. For participants on treatment, decline was slowed in episodic memory, semantic memory, executive function, and global cognition (de Jager et al., 2012). There was a clinical effect for those who started the study with Hcy >13 μmol/L, in that the Clinical Dementia Rating (CDR) Scale overall scores improved significantly, such that participants had a 6-fold chance of reverting to a normal CDR score = 0 on treatment compared with placebo (de Jager et al., 2012). Fig. 1 depicts a simplified pathway of the effect of treatment as described by Douaud et al. (2013). A Bayesian network analysis suggested the following causal chain of events: treatment led to a change in plasma concentrations of vitamin B12 and folate, with only vitamin B12 appearing to play a role in modifying Hcy levels, and lowering of Hcy levels caused a slowing in gray matter atrophy, which, in turn, led to a modification of the CDR (sum of boxes) (model fit, χ^2, $p = 0.64$) (Douaud et al., 2013).

3. Critical thresholds of Hcy and brain atrophy

Critical to these treatment outcomes were the threshold levels of Hcy and rate of atrophy on cognitive decline. In terms of Hcy, the

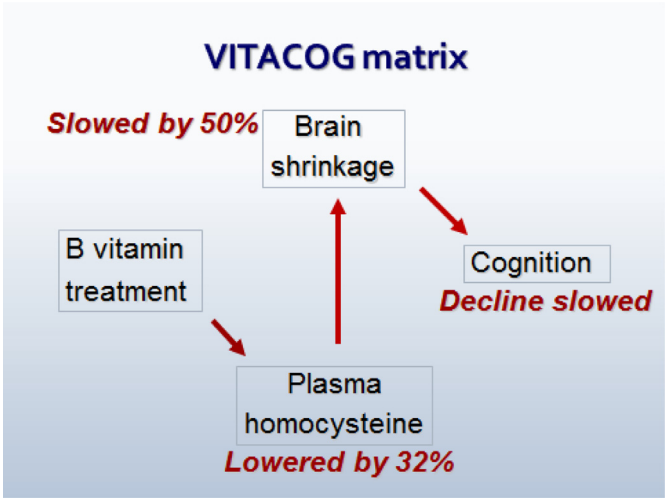

Fig. 1. Simplified model of causal links determined by Bayesian network analysis of B-vitamin treatment and changes in homocysteine, gray matter volume, and cognitive performance over 2 years (as described in Douaud et al., 2013).

critical thresholds were >11.3 and 13 μmol/L. Participants with baseline levels below these thresholds (in other words, those with normal levels reflecting no B-vitamin deficiency) did not benefit from high-dose treatment >2 years. The extra B vitamins did not improve their cognition at the group level. It was only the participants with elevated Hcy who showed a difference in their cognitive scores that was dependent on the B-vitamin treatment. Those without treatment declined significantly more than those on treatment.

3.1. Further analyses

To describe this critical level of brain shrinkage on cognitive performance, novel data analysis comparing the measures of brain shrinkage at the end of the VITACOG trial with certain cognitive scores collected at a number of time points over the 2 years has been performed. The rate of brain volume change was measured as described previously (Smith et al., 2010), and the cognitive measures (Telephone Inventory for Cognitive Status—Modified [TICS-M] and Hopkins Verbal Learning Test—Delayed Recall [HVLT-DR]) are described in de Jager et al. (2012) (supplementary appendix). When these measures were analyzed by quartiles for rate of brain shrinkage for all the trial participants with 2 magnetic resonance imaging scans ($n = 168$), it was found that those who had the fastest brain shrinkage showed the most cognitive decline (Fig. 2A and B). Those whose brain shrinkage rate was lowest (in the upper 2 quartiles) showed no loss of cognitive performance over 2 years on 2 tests, the HVLT-DR and the TICS-M, tested multiple times during the trial. For the HVLT-DR, those in the bottom 2 quartiles did show decline, especially those in the bottom quartile where the atrophy rate ranged from 1.27% to 3.32% per year. The cognitive decline was most apparent after 12 months. Thereafter, the percentage correct answers on the HVLT-DR dropped to as low as 35%, compared with those in the upper 2 quartiles who scored about 70% correct answers. Interestingly, those in the bottom quartile of atrophy started the study with low cognitive performance (about 45% correct answers) (Fig. 2A). Similarly, for the TICS-M, a global cognition test, those in the upper quartile of brain shrinkage improved from 65% to 70% correct responses over the 2 years, whereas the bottom quartile dropped from 60% correct to about 58%; so, the difference in performance between the groups changed from 5% at baseline to 12% after 2 years (Fig. 2B). Thus, one can conclude that there is a critical level of brain shrinkage, which when reached, results in cognitive decline, most marked in episodic memory performance. Thus, there may be a certain window of opportunity to capture and reverse cognitive decline. B-vitamin trials in those with more advanced AD pathology may have been unsuccessful because of the severity of brain atrophy already reached (Aisen et al., 2008); in the latter trial, a significant slowing of cognitive decline was found only for patients with mild AD. Thus, early intervention is indicated. Further support for this concept comes from a clinical trial (Eastley et al., 2000) showing that 66 patients with dementia and low B12 did not improve their cognitive scores after 7 months of B-vitamin treatment. However, 21 patients with early cognitive impairment and low B12 did improve their verbal fluency scores after 9 months of treatment.

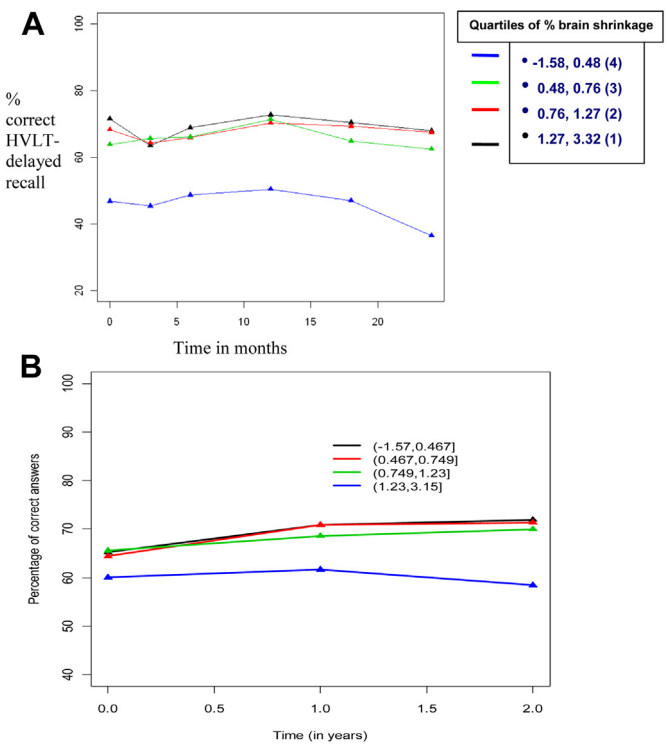

Fig. 2. Relation between quartiles of percentage rate of atrophy per year and (A) Hopkins Verbal Learning Test, Delayed Recall (HVLT-DR) performance at 6 time points and (B) Telephone Inventory for Cognitive Status, Modified (TICS-M) performance at 3 time points over 24 months.

4. Dosages, dietary sources, and cautions with folic acid fortification

Although high doses of B vitamins have been used in RCTs (e.g., 800 μg B12) and US recommendations for those >50 years are to supplement with 250 μg per day, studies with B vitamins from the diet indicate that between 6 and 10 μg B12 per day is sufficient for those not suffering from B12 deficiency (Vogiatzoglou et al., 2009). Beyond this amount, a saturation point is reached, and absorption is limited. This is still well above the amount of B12 in many supplements where only 2 μg is included as the recommended daily allowance in countries such as the United Kingdom. To obtain the 6–10 μg amount of vitamin B12 from the diet, foods rich in animal protein are the best source. A study to determine the bioavailability of B12 from animal foods including meat, fish, dairy products (milk, cheese), and eggs concluded that the best bioavailable sources were in milk and fish (Vogiatzoglou et al., 2009). Fish has not been found to lose substantial amounts of B12 on cooking, whereas heating milk reduces B12 by about 30% and similarly for cooked meat. Milk contains 0.2–0.4 μg/100 g B12. The body absorbs up to 1.5–2 μg per meal of B12 before the active transport mechanism is saturated. Thus, half a liter of milk at 1 meal would supply 1–2 μg B12.

Low-dose vitamin B12 supplements (e.g., 0.2–2.0 μg per pill per day) are ineffectively absorbed and show no effect on plasma levels. However, supplements are a suitable alternative to dietary sources for those groups or populations vulnerable to deficiency, including older adults (because absorption from the gut is often poor), pregnant or lactating mothers, vegetarians, and those in countries with low animal-derived staple diets and those using folic acid fortification. Passive absorption of about 1% of supplemented B12 occurs and a dose of 500 μg per day would give an extra 5 μg to the body even in those with no functional active mechanism. A study of people on a macrobiotic diet revealed that 51% were B12 deficient and 30% had raised MMA levels (Miller et al., 1991).

4.1. Folic acid fortification

Folic acid fortification of foods was introduced as a measure to prevent neural tube defects in the developing fetus and thus is targeted at females of child-bearing age and pregnant women. However, folic acid fortification is not always beneficial, especially

for the older adult population (Smith et al., 2008) and to those suffering from pernicious anemia, which is quite common (Morris, 2012b). The folic acid will reduce Hcy levels but mask B12 deficiency, which if not treated will result in peripheral neuropathy, tiredness, lack of energy, and permanent cognitive deficits. A vitamin B12 level <150 pmol/L is considered deficient and a sign of anemia. People with this condition are often treated with intramuscular B12 injections (1000 μg) daily initially, followed by monthly boosters that effectively reverse the condition and improve memory performance. A combination of injections and supplements has also been found to be highly effective (Butler et al., 2006). In some cases, because of inflammatory factors and oxidative stress associated with elevated Hcy, particular forms of vitamin B12 supplements such as glutathionyl-cobalamin may be more beneficial than others such as cyanocobalamin (Birch et al., 2009) in combination with *N*-acetyl-L-cysteine.

Studies of the effects of high folic acid supplementation have revealed deleterious effects on cognition (Smith, 2007). The National Health and Nutrition Examination Survey study showed that if both B12 and folic acid are at normal levels, there is no deficit in cognition. With normal B12 and high folate levels (>59 nmol/L), cognition improved and reduced the odds of anemia by 0.5. However, low B12 (<148 pmol/L) with normal folate increased the odds ratio for anemia to 2:1 and for cognitive impairment to 1.7:1. But the most dramatic effects were seen with low B12 and high folate where the odds ratio for anemia and cognitive impairment rose to 5:1 (Morris et al., 2007). Thus, food folic acid fortification for those with B12 deficiency is potentially harmful. A similar effect on cognitive decline over 8 years for those with low B12 levels who took folic acid supplements was shown in the Framingham study (Morris et al., 2012). Folate occurs in many foods including dark green, leafy vegetables, citrus fruits and juices, whole grains, poultry, liver, and shellfish. Thus, for vegetarians and older adults, there should be no need for folic acid supplements.

5. Summary

Elevated Hcy is a risk factor for brain atrophy, cognitive decline, and AD. Hcy can be lowered with B vitamins that are important cofactors in the methylation cycle of Hcy. Together, these cofactors play a role in DNA repair and integrity of the neural membranes; thus, deficiencies will result in damage and brain atrophy.

Treatment with B vitamins can reduce the rate of brain shrinkage in older adults, especially in those with elevated Hcy. The treatment can also delay cognitive decline if taken long term (over 1 year) in those with high Hcy levels. Treatment is likely to be more beneficial in those whose brain shrinkage has not yet reached critical levels and in those who do not yet have dementia.

Disclosure statement

The author has no actual or potential conflicts of interest.

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