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Review Article

The folate puzzle. Part III: folate and cognitive function – medicine or myth?

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Abstract

Increasing attention is being dedicated towards the lowering of plasma homocysteine concentrations in order to preclude cognitive impairment in the elderly based on various observational studies that have shown an inverse relationship between homocysteine levels and cognitive function. Folic acid supplementation reduces the plasma concentration of homocysteine in an enzymatic process that uses vitamin B_{12} as cofactor. Deficiencies in folic acid (folate) and/or vitamin B_{12} can lead to neurological impairment, including cognitive decline. However, in view of inconsistent clinical results, Cochrane Database reviewers concluded in 2003 from observational studies that there was no clear evidence for beneficial effects of folic acid. Nevertheless, two large, long-term, longitudinal population studies, the Framingham and the Maine-Syracuse studies, more recently found an inverse relationship between homocysteine levels and a broad range of cognitive abilities for individuals aged 60 years or over. In this context, a double-blind, placebo-controlled, randomized Dutch study lasting over 3 years provided clear evidence that those participants with normal vitamin B_{12} status taking folic acid supplementation scored significantly better on various cognitive domains that usually decline with age. Although encouraging, this finding needs to be confirmed by other studies, preferably carried out in populations with a relatively high prevalence of dementia.

Introduction

Improvements in the diagnosis and treatment of illness are presently the major factors leading to a growing elderly population. The population of the United States has more than tripled during the 20th century. In the year 2000, the U.S. population amounted to 281 million and projections suggest it will reach 308 million in 2010 (www.cencus.gov). In addition, recent data reveal an increase in adults aged over 60 years. The 'greying' of Europe is illustrated by the fact that around 2050, people over 60 will constitute 40% of the total population (source: Eurostat). The World Health Organization (WHO) reported that in the year 2000 about 10% of the world's population was 60 years of age or older and that this figure would rise to over 20% by 2050 (www.who.int/gender/documents). These numbers have made public health politicians express their concern about diseases occurring especially in the elderly, such as dementia. Indeed, it has been estimated that the number of patients suffering from dementia will double every 20 years (1, 2).

The most common form of dementia is Alzheimer's disease (> 50%), followed by non-Alzheimer's disease and vascular dementia. Clinical characteristics include a global progressive decline of cognitive function, impaired daily activities and psychological disorders.

Various observational studies have shown an inverse relationship between plasma homocysteine concentrations and cognitive function. Plasma homocysteine levels can be reduced by folic acid and vitamin B_{12} intake. Thus, if homocysteine represents an independent risk factor, the risk of dementia may be modifiable.

This article will focus on the breadth of evidence for the effect of folic acid and vitamin B_{12} on cognitive function in subjects over 60 years of age. However, we shall first briefly review the biochemical pathways of homocysteine and dwell on homocysteine-related aspects of neuronal dysfunction and damage.

Biochemical pathways of homocysteine

Homocysteine is a sulfur-containing amino acid and its metabolic pathways are closely related to those of folic

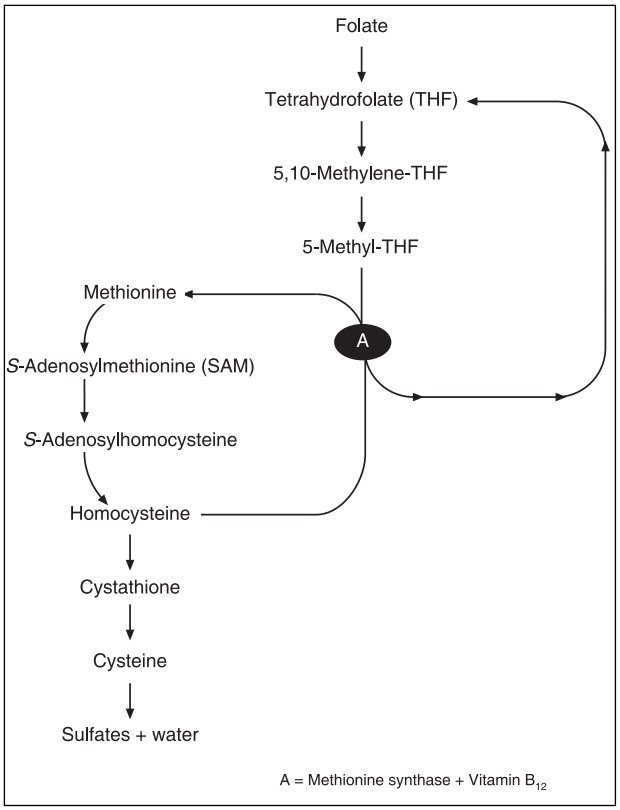

Fig. 1. Simplified representation of homocysteine metabolism in relation to the methionine cycle and the folate cycle.

acid (often referred to as vitamin B_9 or B_{11}) and vitamin B_{12}. It originates from dietary methionine, which is converted to *S*-adenosylmethionine (SAM), the universal methyl group donor for cellular biochemical reactions. The methylation process converts SAM to *S*-adenosylhomocysteine, which is the biochemical precursor of homocysteine. This methionine cycle shares part of its reaction process with the folate cycle: to save methionine for the body, homocysteine can be converted to methionine using methyltetrahydrofolate, which is generated from tetrahydrofolate (Fig. 1). This shared reaction is catalyzed by methionine synthase, with vitamin B_{12} as cofactor. These reaction cycles demonstrate why folic acid intake (resulting in the biologically active compound tetrahydrofolate) results in a reduction of plasma homocysteine levels and why vitamin B_{12} is an essential compound in this process.

Hyperhomocysteinemia

Under normal physiological conditions, the homocysteine concentrations in plasma are below 15 μmol/l, although elderly people often have higher physiological values (3). Levels up to 50 μmol/l are classified as mild, levels up to 100 μmol/l as intermediate and above 100 μmol/l as severe hyperhomocysteinemia (HHcy) (4). Various lifestyle factors, notably physical activity, are inversely related to homocysteine levels, although smoking has no influence. Ethnic origin is not related to homocysteine levels (5). However, a Mediterranean diet including fruits and vegetables may lower homocysteine concentrations (6). Noteworthy is the fact that various drugs, such as lipid-lowering drugs, antiinflammatory agents, insulin and diuretics, affect homocysteine levels (7).

Elevated homocysteine levels are also found in patients suffering from malignancies, especially colorectal cancer (8). Higher homocysteine levels are found in individuals with mutated methylenetetrahydrofolate reductase (*MTHFR*). This contributes to greater plasma homocysteine levels as the *MTHFR* C677T genetic polymorphism codes for a thermolabile variant with reduced enzymatic activity. The heterozygous point mutation in *MTHFR* is usually not associated with HHcy, whereas the homozygous mutation definitely leads to elevated plasma homocysteine levels (9).

The nervous system and B vitamins

Deficiencies in folic acid and/or vitamin B_{12} can lead to hematological disorders and/or diseases of the nervous system. Hematological consequences include mainly megaloblastic anemia, a disorder characterized by large immature and dysfunctional erythrocytes and hypersegmented nuclei in the neutrophils. Neurological impairment associated with folic acid and/or vitamin B_{12} deficiency can occur in patients with or without anemia. Interestingly, vitamin B treatment of these patients corrects the anemia but does not stop the progression of neurological symptoms. The most common neurological findings are peripheral neuropathy (numbness or paresthesias) and gait abnormalities. Neuropsychiatric and neuropsychological manifestations include affective disorders, depression and cognitive changes.

The above-mentioned disorders are most likely due to impairment of DNA synthesis. In the folate metabolic cycle, tetrahydrofolate participates in purine synthesis and in the conversion of deoxyuridylate to deoxythymidine. In the phosphorylated state the latter compound is used for DNA synthesis. Vitamin B_{12} deficiency leads to inadequate deoxythymidine triphosphate and consequently impaired synthesis of DNA. (Under these conditions, protein synthesis is not disturbed, as RNA does not contain the thymidine compound. The ongoing protein synthesis results in macrocytosis.)

Folate and/or vitamin B_{12} deficiency is associated with SAM deficiency and abnormal methylation processes. DNA fidelity is strongly dependent on DNA methylation. Abnormal gene expression affects serotonin, norepinephrine and dopamine synthesis, and consequently neuronal functions (10). (Functional aspects of neurotransmitters have been associated with depression, which makes SAM a candidate antidepressant.) Furthermore, neuronal function may be impaired due to increased microvascular permeability under the influence of HHcy. Mouse experiments have suggested that this increased endothelial cell permeability occurs through inhibition of γ-aminobutyric acid (GABA) receptors. Homocysteine competitively

binds to these receptors, alters the biological structure and has a toxic effect on the cerebral endothelium by increasing metalloproteinase activity (11). This also brings about homocysteine-induced stress and reduces the bioavailability of nitric oxide, a compound essential for vascular tone (12). Additionally, high homocysteine levels promote neurotoxicity through oxidative damage induced by β-amyloid (Aβ) peptide. It is known that neurons are vulnerable to death induced by the Aβ peptide. This is the result of Aβ-induced oxidative modification of DNA bases and resulting DNA damage (13).

Taken together, folic acid and/or vitamin B_{12} deficiency and the consequent rise in homocysteine levels lead to abnormal methylation. This results in impaired DNA repair and DNA synthesis. The various mechanisms suggested for HHcy-promoted dementia, including endothelial dysfunction, neurotoxicity, neuronal DNA damage, neurotransmitter abnormalities and Aβ-induced oxidative damage, all share a common factor: methylation. The DNA damage response is strongly dependent on proper methylation of genes encoding for the DNA repair machinery.

Postmitotic neurons must survive for the entire life of the organism. Neuronal cells, except neurons of the hippocampus, cannot regrow after damage. Recently, Zhang *et al*. (14) stated that the telomeres at the chromosomal ends should remain stabilized to increase the neuronal lifespan. Telomere maintenance occurs through specialized proteins, particularly those of the phosphatidylinositol 3-kinase (PI3K)-related kinase family. In addition, telomeric binding factors such as TRF2 are involved in telomere maintenance. Dysfunction of TRF2 causes exposure of the telomere ends and activates DNA response mechanisms (15). Thus, two major cellular processes safeguard neuronal genomic integrity, *i.e.*, DNA repair and telomere maintenance. These apparently divergent physiological responses may have some cross-talk, as they both pursue the same goal.

Thus, counteracting neuronal apoptosis is presently considered a promising approach for preventing neurodegenerative diseases. In the context of this article, it is intriguing that HHcy or folate and/or vitamin B_{12} deficiency promotes apoptosis and neuronal death (16).

Homocysteine and cognitive decline

In his historic overview, Reynolds (17) sketched the clinical and biochemical developments that led to a better understanding of the relationship between folate, vitamin B_{12} and hematological and nervous system disorders. The author mentioned the fact that only in the final third of the 20th century did assays for folate, vitamin B_{12} and homocysteine become available. This paved the way for further research into the roles of these compounds in various diseases, notably neurological disorders.

Indeed, many studies have provided evidence for an association between homocysteine and cognitive impairment. Many of these investigations are observational studies (see BOX) and their heterogeneity and limited scope do not allow straightforward conclusions. In fact, the results of studies reported between the mid-90s and the beginning of the new millennium are inconsistent and inconclusive. For example, Jelinic *et al*. (18) found no evidence for a vitamin-related memory deficit in a population study of 698 older adults with a mean age of 68.7 years. In a 6-year reassessment study in 137 normally aging people, aged 66-90 years, La Rue *et al.* (19) found only a weak association between vitamin status and cognitive function. Similarly, Wahlin *et al.* (20) concluded from a population-based study that vitamin B_{12} levels and folate status may only affect memory performance in individual cases. On the other hand, positive associations between homocysteine, vitamin B and cognitive performance were reported by several other authors. Duthie *et al.* (21) reported an association for these parameters in a cohort study in 331 elderly people. Morris *et al*. (22) investigated a group of people 60 years of age and older and found that HHcy was related to poor performance in short-delay recall tests. However, this association was only partially dependent on folate status. A more recent study from the same institution performed in 1,302 seniors confirmed that low vitamin B_{12} status and high folate status were associated with cognitive impairment and that high serum folate in the presence of normal vitamin B_{12} status protected against this (23). An Italian study reported on a population of 1,353 healthy participants 65 years of age and older and concluded that elevated plasma homocysteine levels were associated with cognitive impairment (23). A few years earlier, however, scientists from the same institution found no biologically important relationship among homocysteine, vitamin B and cognitive skills (24).

Based on these inconsistent results, it is not surprising that Cochrane Database reviewers concluded in 2003 that there is no clear evidence for beneficial effects of folic acid (750 μg/day) on cognition, although folic acid plus vitamin B_{12} was effective in reducing serum homocysteine concentrations (25).

A study of special interest is the Framingham Study, which began in 1948 with 5,209 adults living in the town of Framingham, Massachusetts. Although originally set up to evaluate risk factors for heart disease, this longitudinal study has drawn the attention of many medical disciplines. Using data collected between 1992 and 2002, investigators found an inverse relationship between homocysteine levels and a broad range of cognitive abilities for individuals aged 60 years or over. Such associations were not found for younger participants. As far as the vitamin status was concerned, only vitamin B_{12} was partially related to cognitive performance, although the involvement of other vitamins could not be excluded. The authors (26) stated that early preventive interventions may be important, as the observed relationship occurs beyond middle age. Similar results were obtained from another population study, the Maine-Syracuse Study. Plasma levels of homocysteine were inversely correlated with multiple domains of cognitive function, and folate and vitamin B_{12} were positively correlated with cognitive performance (27, 28).

Interventional studies

From the results of observational studies, an important question emerges: would lowering plasma homocysteine concentrations improve cognitive function in older adults? The proper approach to this issue is to conduct a long-lasting double-blind, randomized, preferably placebo-controlled trial. In this context, it is interesting to note that, on the basis of two pilot studies, it was concluded by Malouf *et al.* (25) that vitamin B treatment in patients with dementia did not significantly improve their cognitive function, although homocysteine levels were successfully lowered. This conclusion was underscored more recently in a summary of results obtained from randomized, controlled trials of vitamin B supplementation in various combinations (29). The authors of this systematic review mention that 202 articles were retrieved from the literature search, but only 14 randomized, controlled trials met the criteria for adequate scientific value. Overall, the studies were "of low quality and limited applicability" due to a lack of blinding and incomplete reporting. Their general conclusion was that there is not yet adequate evidence that this type of supplementation has an effect on cognitive function in people with normal or impaired cognitive performance. There was one exception: people with cognitive impairment and low serum folate levels may benefit from folic acid supplementation. This conclusion was, however, obtained on the basis of limited data, *i.e.,* three relatively short trials with only 39 participants.

A far more firm conclusion can be drawn from a recent article published by Durga *et al.* (30). This excellent study (as part of the Folic Acid and Carotid Intima-Media Thickness [FACIT] trial) aimed to assess the effect of folic acid supplementation on cognitive performance in men and women aged 50-70 years with elevated plasma homocysteine and normal vitamin B_{12} levels. In this Dutch randomized, double-blind, placebo-controlled trial, 818 participants received 800 μg/day folic acid or placebo for 3 years. The serum folate concentrations and plasma homocysteine concentrations increased by 576% and decreased by 26%, respectively, compared to in participants taking placebo. The change in cognitive function after 3 years was measured by memory, information-processing speed and sensorimotor speed, domains that usually decline with age, and the folic acid group scored significantly better than the placebo group. It is of interest that the effect of folic acid supplementation was not modified by the initial folate status or the *MTHFR* C677T genotype. These findings are in contrast with the results of other studies for a number of reasons: the study population was relatively large, the supplementation lasted for a relatively long time and –last but not least– the population under study did not include participants with impaired cognitive function at the start of the trial.

Some considerations

The most recent data strongly suggest a direct association between folic acid and cognitive function in elderly people. This is an encouraging finding which, if confirmed by other studies, has huge social and clinical consequences. Even a modest benefit will have a considerable effect on the quality of life of elderly people and on the economic burden for society. Moreover, any effective preventive measures or treatment possibilities will have an enormous impact on (future) healthcare systems.

From the foregoing, the question arises as to whether homocysteine levels are a risk indicator, a risk factor, or both. Regardless of the answer, lowering homocysteine levels by folic acid supplementation has demonstrated a significant effect on cognitive function in elderly patients. This indicates that further epidemiological research is necessary. A long-term prospective study in middle-aged people would further elucidate the purported preventive role of folic acid. Preferably, such long-term studies should be carried out in populations with a relatively high prevalence of dementia. Of primary importance is the standardization of scoring methods for cognitive function and of treatment schedules, notably with or without vitamin B_{12}. Of particular interest would be to study the folic acid effect on homocysteine levels in the homozygous 677 CT genotype and its relationship with cognitive decline in the elderly. Is folic acid supplementation able to preclude or even reverse cognitive deterioration in this population group?

Furthermore, imaging studies of the brain deserve attention, especially those using positron emission tomography (PET) scans. This technique employs radiolabeled agents to visualize disease and in particular the associated abnormal biochemical processes. PET scanning has been described as '*in vivo* biochemistry'. The imaging probe ^{18}F-FDDNP, a naphthalene-based radiofluorinated agent, binds to tau neurofibrillary tangles and Aβ plaques, brain lesions typical in Alzheimer's disease. In this way, PET studies allow the three-dimensional *in vivo* mapping of pathological areas, providing an original method for diagnosis and monitoring disease development (31). The distribution of ^{18}F-FDDNP in the brain corresponds well with the distribution of tangles and plaques, as seen in postmortem studies of brains of Alzheimer's patients. Using global binding values, PET studies can differentiate individuals with mild cognitive impairment from those with Alzheimer's disease and those with no loss of cognitive function (32). In addition, the pattern of radiotracer accumulation in the brain cortex correlates well with, among other things, memory scores (33). It is therefore likely that such imaging studies will play a key role in future trials, as PET is an excellent modality for quantitatively assessing the relationship between vitamin B supplementation and changes in cognitive function. This will help to further explore the hypothesis that elevated plasma homocysteine levels contribute to a higher risk of cognitive impairment.

Dedication

E.K.J. Pauwels dedicates this paper to Doctor Louk Lindner who taught him the first steps on the ladder of science and scientific writing.

BOX: Epidemiological studies

In clinical medical research, epidemiology investigates the causes and patterns of disease. This includes the study of the effect of treatment, *e.g.,* a new medication. To study the effects of such medication, information can be collected from clinical trials in which participants take the medication under study or a placebo, thereby ensuring that equivalent groups are compared. This type of research is important to develop evidence-based medicine, which aims at applying qualified methods in medical practice with the highest chance that the best possible outcome is obtained according to the state of the art. In order to gather such knowledge, epidemiologists employ various study designs. Those most often used are observational studies and randomized clinical trials.

Most epidemiological studies are observational, as they are cheap and relatively easy to perform. In 1965, W.G. Cochrane, one of the fathers of biostatistics and epidemiology, defined the observational study as an empiric comparison of treated and control groups in which the objective is to elucidate cause-and-effect relationships. A typical example of an observational study is a case-control study in which researchers recruit a group of people with disease and a group of people without disease. By comparing these groups, it is possible to identify factors that may have caused or contributed to a well-defined medical condition. Although the results of a case-control study have limited scientific value (the factor may not have caused the disease), important medical discoveries have been made using this study design. A well-known example is the case-control study that demonstrated the link between smoking and lung cancer.

Another type of observational study is the cohort study. In this study design, the researchers recruit a number of people who do not have the disease in which the researchers are interested, *e.g.,* coronary artery disease (CAD). This type of study usually takes a number of years and at the end of the study it is recorded who has developed CAD and who has not. By comparing factors such as diet or lifestyle, the cohort study will identify risk factors. Cohort studies are very useful to find out more about risk factors, but they take a long time and require much effort to maintain a database.

After observational studies, epidemiologists use randomized trials, which usually provide more compelling results. Starting from a hypothesis, the randomized, controlled study may indicate that the medication has the expected effect. In this type of study, each participant receives either the medication under study or a placebo, all in a randomized manner. If the study design is blind, the participants and the physicians involved do not know which patient receives the medication and which patient receives the placebo. Such designs avoid every possible bias from the side of the reporting participant and the reporting physician.

Epidemiological studies require statistical analysis to evaluate the significance of the results obtained. Statistical analysis is also necessary to correct for confounding factors. In general, the larger the number of participants, the greater the reliability of the outcome. This is often referred to as the 'power of the trial'. In designing a clinical trial, this power should be taken into account beforehand. Needless to say, high-powered studies require many participants (often more than 1,000) and take a long time (often on the order of several years).

Author disclosure

E.K.J. Pauwels is Professor of Radiological Sciences, the Leiden University Medical Center, Leiden, The Netherlands, and Socrates Professor of Nuclear Medicine at the University of Pisa Medical School. H.S. Balter is Professor of Pharmaceutical Sciences, the University of Uruguay, Montevideo, Uruguay.

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