a transference to the agent itself that perhaps enhanced response. Finally, since all patients tested positive for at least one MTHFR polymorphisms, which confers defective folate metabolism, and since there is synergy associated with the dual presence of C677T and A1298C, many of ourpatients would be expected to respond robustly to the compound containing three forms of highly bioavailable folate. Therefore, we cannot extrapolate these findings to individuals who test for normal variants of C677T and A1298C.

In conclusion, our findings help to confirm the HCY basis of MDD. MTHFR polymorphisms have long been established as a risk factor for depression, but they may also be considered markers for the presence of coexisting polymorphisms associated with HCY metabolism and suboptimal monoamine production. The long held belief that the serotonin transporter protein (SERT) is genetically different in individuals with MDD has recently been refuted. Thus, addressing the HCY theory of depression clinically, by circumventing all possible genetic polymorphisms associated with elevated HCY levels, is, in effect, addressing the root cause of depression.

Since higher than normal HCY levels are associated with many neurodegenerative disorders and congenital conditions, the use of reduced B vitamins for neuroprotection and prenatal implications are obvious areas of future study.

References

1. Carney MWP, et al. Red cell folate concentrations in psychiatric patients. J *Affective Disorders*. 1990;19:207-13.
2. Penninx BWJH, et al. Vitamin B12 deficiency and depression in physically disabled older women: epidemiological evidence from the women's health and aging study. *Am J Psychiatry*. 2000;157:715-21
3. Elby EM, et al. Folate status, vascular disease and cognition in elderly Canadians. *Age Aging*. 1998;27:485-91.
4. Tiemeier H, et al. Vitamin B12, folate, and homocysteine in depression: The Rotterdam Study. *Am J Psychiatry*. 2002;159(12):2099-2101.
5. Mitchell ES, et al. B vitamin polymorphisms and behavior: Evidence of associations with neurodevelopment, depression, schizophrenia, bipolar disorder and cognitive decline. *Neurosci and Biobehav Rev*. 2014;47:307-20.
6. Folstein M, et al. The homocysteine hypothesis of depression. *Am J Psychiatry*. 2007;164(6):861-7.
7. Lok A, et al.Interaction between the MTHFR C677T polymorphism and traumatic childhood events predicts depression. *Translational Psychiatry*. 2013;3:e288.
8. Carney MWP, Sheffield BF. Associations of subnormal serum folate and vitamin values and effects of replacement therapy. *Jl Nervous and Mental Dis*. 1970:150(5):329-417.
9. Reynolds EH, et al. Folate deficiency in depressive illness. *Br J Psychiatry*. 1970:
10. Godfrey PS, et al. enhancement of recovery from psychiatric illness by methylfolate. *Lancet*. 1990:336(8712):392-95.
11. Troen AM, et al. Unmetabolized folic acid in plasms is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutrition*. 2006:136(1):189-94.
12. Reynolds EH. Benefits and risks of folic acid to the nervous system. *J Neurol Neurosurg Psych*. 2002;72:567-71.
13. US Food and Drug Administration. Package insert for Enlyte capsules. http://67.210.119.235/~enlyterx/wp-content/uploads/2015/01/EnLyte_PI.pdf
14. Tsai MY, et al. Polygenic influence on plasma homocysteine: association of two prevalent mutations, the 844ins68 of cystathionine β-synthase and A2756G of methionine synthase, with lowered plasma homocysteine levels. *Atherosclerosis*. 2000;149(1):131-

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Article

Reduced B Vitamin Therapy in MTHFR C677T/A1298C Patients with Major Depressive Disorder – Clinical Response Correlates with Homocysteine Reduction: A Double-Blind, Placebo-Controlled Study

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Objective: This study was designed to evaluate the efficacy and safety of reduced B vitamins as monotherapy in adults with major depressive disorder (MDD) who were also positive for at least one of the common MTHFR polymorphisms associated with depression: C677T and/or A1298C. The study further tested the hypothesis that reduced (metabolized) B vitamins will reliably lower plasma homocysteine (HCY), and therefore CNS homocysteine. These findings have implications beyond MDD, as elevated HCY levels are not only associated with depressive disorders, including postpartum depression, but numerous other illnesses and conditions, including cognitive decline in aging, the risk of developing dementias, cerebrovascular events, atherosclerosis, Parkinson's, autism spectrum disorders, and conditions as diverse as neural tube defects, repeated miscarriages, and neuropathic pain.

Method: A total of 330 adult patients with MTHFR C677T and/or A1298C polymorphism who met the Diagnostic and Statistical Manual V criteria for MDD without psychosis were enrolled in the trial. 160 were randomized to receive placebo, while 170 received a gel capsule containing a combination of reduced B vitamins (B1, B2, B6, B12, and three forms of B9) and micronutrients designed to lower HCY in the CNS. Specifically, the active ingredients included citratede folic acid, folinic acid (leucovorin), l-methylfolate magnesium, thiamine pyrophosphate, flavin adenine dinucleotide, pyridoxal 5—phosphate, and adenosylcobalamin, other b vitamin coenzymes, mineral cofactors, betaine, and omega 3s. The trial was conducted between 08/01/2014 and 04/03/2015. MTHFR status was determined initially, and plasma HCY levels were drawn at baseline and at eight weeks for all subjects. Subjects were screened for potential side effects and adverse events at weeks two, four, and eight. The MADRS was used to determine efficacy for MDD.

Results: All subjects met the criteria for MDD and were positive for the MTHFR polymorphisms associated with depressive disorders. While 159 of 170 B vitamin patients completed the 8-week study, 123 of the 160 patients receiving placebo were full completers. Of the 159 patient completers who received reduced B's, 131 (82.4%) showed a reduction in plasma homocysteine (an average of 2.7 µmol/L from a baseline of 9.6 µmol/L, $p<0.001$), while 28 (17.6%) showed no significant change in plasma HCY. The 123 patients who received placebo demonstrated a small elevation in HCY levels (of 0.4 µmol/L). Active treatment patients demonstrated, on average, a 12 point reduction on the MADRS by week 8, and 42% of vitamin monotherapy patients had achieved full remission by the end of the trial ($p<0.001$). No side effect was significantly different between placebo and active treatment groups. No patients experienced mania in either group.

Conclusion: A combination of reduced B vitamins and micronutrients in capsule form, indicated for conditions associated with lower than optimal folate levels in the CNS and resulting hyperhomocysteinemia, when used in the treatment of MDD in MTHFR positive patients, significantly separated from placebo by week two of treatment on the MADRS rating scale, and separation expanded throughout the trial. Baseline Montgomery-Asberg score was reduced by 12 points on average in the treatment group with 55 of 131 (42%) achieving remission by the week eight visit. Further, treatment response was associated with a significant reduction in HCY levels, 32.9% compared to placebo. Patients receiving placebo experienced, on average, a slight, non-significant increase in HCY levels of 0.4 µmol/L. These results confirm the HCY theory of depression and the therapeutic benefit and safety of using reduced B vitamins as monotherapy in depressive disorders particularly in the presence of MTHFR Polymorphisms.

Introduction

Homocysteine metabolism, or the carbon-1 cycle, plays a key role in the synthesis of monoamines by providing for methyl group donation in the production of these neurotransmitters. Reduced, or metabolized B vitamins, are necessary coenzymes in the carbon-1 cycle, and in various other enzymatic steps involved in monoamine synthesis. In general, B vitamins enter the body as pro-drugs, and must be metabolized to their active “coenzyme” forms. Impaired B vitamin metabolism will result in a deficiency of coenzymes, a subsequent rise in HCY, and less than optimal monoamine production.

Various studies have correlated impaired B vitamin metabolism with elevated homocysteine levels and resultant depressive disorders. (1,2,3,4,5) Mutations, or even minor variations in the genes coding for enzymes necessary for B vitamin metabolism can lead to inadequate coenzyme production and lower than optimal levels of serotonin, norepinephrine, and dopamine. The most common of these polymorphisms are the C677T MTHFR variants, of which there are at least 40, although they often coexist in the presence of other less studied polymorphisms involved in metabolizing B12 and B6. In summary, the homocysteine theory of depression argues that these genetic variants result in a deficiency of metabolized B vitamins, and thus, inadequate coenzymes for HCY reduction, elevated HCY levels, suboptimal monoamine production, which can manifest clinically as depression. (6) The earliest elaborations of HCY’s role in depression also listed vascular damage as contributory. Further, new evidence has also supported this genetic basis of depression, as MTHFR polymorphisms are prevalent in those who have depressive disorders after childhood trauma at a higher rate than in those who experience similar traumas and do not suffer depression. (7)

Based on this evidence, B vitamins in various forms have been used as monotherapy and adjunctive therapy in MDD since the 1960’s. (8,9,10) Yet due to the variety of preparations utilized, the diversity of study populations and methodologies, and the lack of clarification regarding the exact role of B vitamins in treatment (as adjunctive or monotherapy), no consensus exists regarding current treatment recommendations. Further, it is only in the last decade that the therapeutic emphasis has been on metabolized B vitamins, as the issue in the vast majority of depressed patients is not their dietary intake, but their metabolism of vitamins that is problematic. Thus, many prior studies were unsuccessful because patients were given high doses of vitamins they simply could not metabolize effectively to the active coenzymes needed, casting doubt on the therapeutic role of B vitamins, and raising legitimate safety concerns regarding administering ineffective high doses of unmetabolizable vitamins. (11, 12)

Our study utilized a combination of the metabolized vitamins and micronutrients necessary for HCY reduction and monoamine production, and this formulation is FDA regulated with USP and indicated for folate deficiencies associated with elevated HCY in the CNS. (13) We also utilized MTHFR polymorphism as evidence of impaired folate metabolism, and further, as a marker for the likely presence of other B vitamin polymorphisms in MDD patients. Thus, utilizing a combination of B’s in reduced form addressed all possible polymorphisms contributing to inadequate monoamine production.

Further, the study was also designed to test the homocysteine theory of depression: i.e., circumventing the patient’s genetic inability to metabolize B vitamins by administering their metabolized form in an optimal preparation for CNS availability, lowering HCY, increasing monoamine production, and allowing for clinical improvement.

Method

Patients:

Patients were recruited using print advertisements and referrals from other professionals. The ages ranged from 18-67, with an average age of 32. Males comprised 42% of the study population, and females 58%. All patients met the DSM-V criteria for MDD without psychotic features, and those with comorbid ADHD or GAD were not excluded. However, exclusion criteria included active substance abuse or dependence, dementia, current psychotic symptoms, suicidality requiring hospital care, and bipolar disorder. Past treatment failures or lack of response to current therapy did not exclude patients. All patients consented to the study voluntarily and understood the possibility of receiving a placebo agent. All patients were instructed to phone immediately if manic symptoms, suicidal intent, or new onset suicidal thoughts occurred.

Study design:

All potential patients were screened for MTHFR polymorphisms, and 330 screened positive for one of the two C677T polymorphisms, and/or one of the A1298C polymorphisms associated with MDD. Baseline homocysteine levels were drawn and repeated at 8 weeks post treatment with either reduced B vitamins or placebo.

The active ingredients in the gel cap dispensed to the treatment group included: three forms of B9 (1mg of citrated folic acid, 2.5 mg of folinic acid, and 7 mg l-methylfolate magnesium), 25 mcg of thiamine pyrophosphate, 25 mcg of flavin adenosine dinucleotide, 25 mcg of pyridoxal 5-phosphate, 50 mcg of adenosylcobalamin, 25mcg of thiamine pyrophospahate, 25mcg of NADH, 500mcg of trimethyl glycine, 1.5mg of AminoFerr, 24mg. of magnesium ascorbate, 1mg of zinc ascorbate, 1mg of L-threonic acid magnesium, 20mg of Sharp’s PS Gold omega 3.

Patients could withdraw at any time. Of the 165 vitamin therapy patients, 159 patients completed the 8-week trial versus 123 placebo-assigned patients. Of the six active treatment patients who withdrew from the study, none did so because if side effects, but due to a move, the feeling they had recovered and needed no further follow-up, or for nonspecific reasons.

Results:

Of the 165 patients randomized to revive reduced vitamin therapy, 159 completed the 8-week trial, while 123 placebo-assigned patients were completers. Of the treatment-assigned patients completing the trial, 131 (82.4%) demonstrated a reduction in Homocysteine levels (from 9.6 at baseline to 7.2 µmol/L at week 8 on average, $p<0.001$). For 28 (17.6%) of active treatment patients, no statistically significant change in HCY was noted. The 123 placebo-assigned patients who completed the study demonstrated a slight increase in homocysteine from baseline, rising on average 0.4 µmol/L.

The entry MADRS score was, on average 27, for all participants. In the active treatment group, separation from placebo was seen by week 2, ($p<0.001$) and by week 8, the average drop in the treatment group was 12 points. Further, 55 of the 131 vitamin therapy completers, or 42%, had achieved full remission by week 8 ($p<0.001$)

Though some patient reported thoughts of death or suicidal ideation prior to entry, no patient in the active treatment group experienced an increase or the new onset of suicidal ideation. Rates of nausea, headache, anxiety, or tremor were all less than 5% in both groups and no side effect occurred at a rate that was statistically different than placebo. No patients in ether group experience mania, hypomania, or psychosis.

Discussion

The homocysteine theory of depression argues that inadequate monoamine production results from a genetic inability to optimally metabolize HCY. Homocysteine metabolism in the CNS can occur via two pathways, the first using reduced B12, betaine, and l-methylfolate as coenzymes to allow for the methylation of precursors to the production of monoamines, while the second, predominately in glial cells, utilizes B6 as and results in antioxidant production (glutathione). B vitamins enter the body as pro-drugs and must be metabolized to their active forms through enzymatic steps. Due to genetic variants, or polymorphisms, in the enzymes responsible for metabolizing B vitamins to their coenzyme forms, many less functional enzymes are possible in our patients. Thus, HCY is less optimally reduced, its levels increase, and lower levels of monoamines result. Further, B1, B2, B3, mineral cofactors, and Omega 3s are essential in various other steps needed or monoamine synthesis.

Though MTHFR polymorphism is the most studied genetic variant resulting in suboptimal vitamin metabolism, a finding in up to 70% of depressed individuals, patients with MDD can present with multiple polymorphisms. A study of non-depressed individuals found that the regulation of HCY was in fact polygenic for over 50%. (14) The possibility of multiple polymorphisms argues for utilizing a cluster of reduced B vitamins for clinical benefit rather than single vitamins.

Our study demonstrated that in individuals with known MTHFR polymorphisms associated with depression, addressing all other possible polymorphisms that may result in inadequate HCY metabolism with reduced B vitamins was superior to placebo in lowering HCY levels, and further, resulted in a statistically significant clinical response for the majority of patients, and complete remission for 42% of the active treatment group. Patients receiving placebo experienced a slight elevation in plasma HCY. We assume that the untreated depression resulted in further and prolonged psychosocial stress, a known cause of HCY elevation. For patients who responded clinically, yet did not demonstrate a reduction of plasma HCY, we observed that they were taking medications known to cause HCY elevations (such as lipid lowering agents or hypogylcemics) or there were lifestyle factors (such as heavy smoking) that may have countered the HCY lowering effects of active treatment in the periphery, yet did not preclude adequate monoamine production centrally.

No side-effect was reported at a greater than placebo rate, no patients converted to mania, and no patients reported new-onset suicidal thinking on either reduced vitamins or placebo.

The limitations of our study include the lack of an active comparator agent, such as an SSRI or an SNRI. Since it is not practical to measure HCY levels in the CNS, we relied on peripheral HCY measures, yet these are believed to approximate CNS levels. Patients also understood they were possibly receiving a natural compound which may have conveyed a sense of safety not associated with typical active drug vs. placebo studies, and may have allowed for a minimization of side effect reporting. Further, the appeal of a natural therapy may have conveyed