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COMMENTARY

Low Vitamin B12 and Parkinson Disease: Potential Link to Reduced Cholinergic Transmission and Severity of Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by prominent motor dysfunction including rest tremor, bradykinesia, and rigidity responsive to levodopa. However, postural instability, freezing of gait, and cognitive impairment are important non–levodopa-responsive symptoms of PD and are significant risk factors for falls, leading to hospitalization, disability, and death.[1] Treatments for key nonmotor symptoms of PD that might improve cognitive decline and postural instability are currently a major gap in PD therapeutics.

Dysfunction of the cholinergic systems in PD is thought to possibly play a contributory role in postural instability and cognitive impairment. Therefore, modulation of cholinergic transmission could improve balance and cognition in these patients.[1] Vitamin B12 is lower in patients with PD compared with controls, and low levels have been associated with peripheral neuropathy, cognitive impairment, and more rapid rate of disease progression in PD.[2-4] Although this relationship does not necessarily mean causality, there are several hypothetical mechanisms by which reduced vitamin B12 may lead to reduced availability of choline as a substrate for cholinergic transmission. Here we will review the proposed pathophysiology of nonmotor symptoms in PD and the potential relationship between vitamin B12 and acetylcholine metabolism. We propose that vitamin B12 supplementation could be considered as an adjuvant approach to improve cholinergic transmission and, potentially, motor and cognitive function in patients with PD.

The Role of Cholinergic Dysfunction in Nonmotor Symptoms of PD

There is evidence for major cholinergic dysfunction early in the course of PD.[5] Acetylcholine is a primary neurotransmitter in many neuronal groups in the central nervous system (CNS). These include neurons of the basal forebrain innervating the cerebral cortex, which modulate attention and sensory processing; neurons in the pedunculopontine tegmental nucleus (PPN) that participate in both thalamocortical arousal and control of muscle tone during locomotion; and giant aspiny neurons in the striatum. In addition to these direct effects, acetylcholine also contributes to control of dopaminergic activity both at the level of the midbrain and the striatum.[6] Lower cholinergic terminal integrity as measured by [(11)C]methyl-4-piperidinyl propionate acetylcholinesterase positron emission tomography (PET) imaging and reduced cholinergic activity assessed with transmagnetic stimulation are associated with slower gait speed compared with patients with isolated striatal nigral degeneration in PD and lower in PD fallers compared with nonfallers.[7-9] *In vivo* cerebral spinal fluid (CSF) studies have shown decreased acetylcholine levels in patients with PD who have postural instability gait disturbance (PIGD) phenotype compared with tremor predominant (TD) phenotypes; however, acetylcholine levels do not appear to be associated with severity of PIGD.[10] Decreased PPN-thalamic cholinergic activity, independent of dopaminergic integrity, is associated with increased postural sway.[11] This may be due to a decreased ability to integrate

sensory cues for position monitoring and attention maintenance secondary to cholinergic dysfunction. Supporting *in vivo* data, pathologic studies of patients with PD have shown that PD fallers have decreased numbers of cholinergic neurons in the PPN when compared with PD nonfallers.[12]

A significant contributing factor to falls in patients with PD is freezing of gait (FOG)—which is an unpredictable—and sudden inability to start or continue walking despite the desire to walk and is exacerbated with decreased attention or during directional change.[13] The pathophysiology of FOG is not completely understood, but cholinergic dysfunction may play a role.[8] Recent evidence indicated that it involves dysfunction in cerebellar circuits centered in the vermis.[13] Although cholinergic input from the PPN to the medulla and spinal cord are, at present, not considered to be part of the central pattern generator for locomotion, they have an important role in regulating muscle tone during locomotion and possibly contribute to modulating attention during locomotion.[8] Further, neocortical cholinergic innervation has shown to be decreased in patients with PD and FOG compared with those who do not have FOG.[14] Overall, current data suggest a role for impaired cholinergic transmission as a contributory factor in FOG by impaired control of postural tone during gait, impaired attentional modulation during gait, or both.

Treatment of patients who have PD with anticholinesterase inhibitors may decrease falls compared with placebo.[15,16] In patients with PD dementia and mild cognitive impairment, cholinergic potentiation with anticholinesterase inhibitors has shown trends toward improved cognition.[17,18] The results of these trials provide evidence for the important role of acetylcholine in both postural stability as well as cognition, which are likely strongly interrelated, as recognition and correction of postural and gait errors are needed for postural stability. Importantly, they highlight the need for additional methods to potentiate cholinergic transmission. However, it remains important to note that cognitive impairment in PD is multifactorial and likely also related to combined underlying microvascular disease, Lewy body deposition, as well as β-amyloid plaques and τ-neurofibrillary tangles in addition to dysfunction of multiple neurotransmitters including dopamine, acetylcholine, norepinephrine, and serotonin.[19] As such, acetylcholine remains 1 part of complex neuronal dysfunction leading to cognitive impairment in PD.

Vitamin B12, Acetylcholine, Homocysteine, and the Transmethylation Cycle

The enzyme responsible for the synthesis of acetylcholine is choline acyltransferase (ChAT), which yields acetylcholine through transfer of an acetyl group to choline.[20] Synthesis of acetylcholine is primarily regulated by the amount of free choline and its presynaptic uptake via a high-affinity choline transporter.[20] The relationship between vitamin B12 and acetylcholine metabolism is complex. This interaction stems from the transmethylation cycle, which depends on vitamin B12 and indirectly regulates the availability of choline. Following vitamin B12-mediated conversion of homocysteine to methionine, methionine is converted to S-adenosylmethionine (SAM), which serves as the major methyl donor for multiple methylation pathways (Figure). Some of these pathways indirectly affect availability of choline. Methylation of nicotinamide to N-methyl-nicotinamide by nicotinamide n-methyltransferase (via SAM) competitively inhibits the efflux of choline out of the CNS.[21,22] In this model, vitamin B12 deficiency may lead to decreased SAM, leading to reduced N-methylnicotinamide and increased choline efflux from the CNS, ultimately lowering acetylcholine levels (Figure).[23]

The second manner in which vitamin B12 relates to acetylcholine levels is through the methylation of homocysteine to methionine. The preferential pathway for methylation of homocysteine to methionine is via the B12-dependent enzyme methionine synthase using the folate derivative, 5-methyltetrahydrofolate (5-THF), as a methyl donor.[24] The folate-B12 pathway also requires vitamin B2 (riboflavin) and vitamin B6 as cofactors involved in the conversion of folate to 5-methyltetrahydrofolate. A secondary pathway for methylation of homocysteine to methionine relies on choline (in

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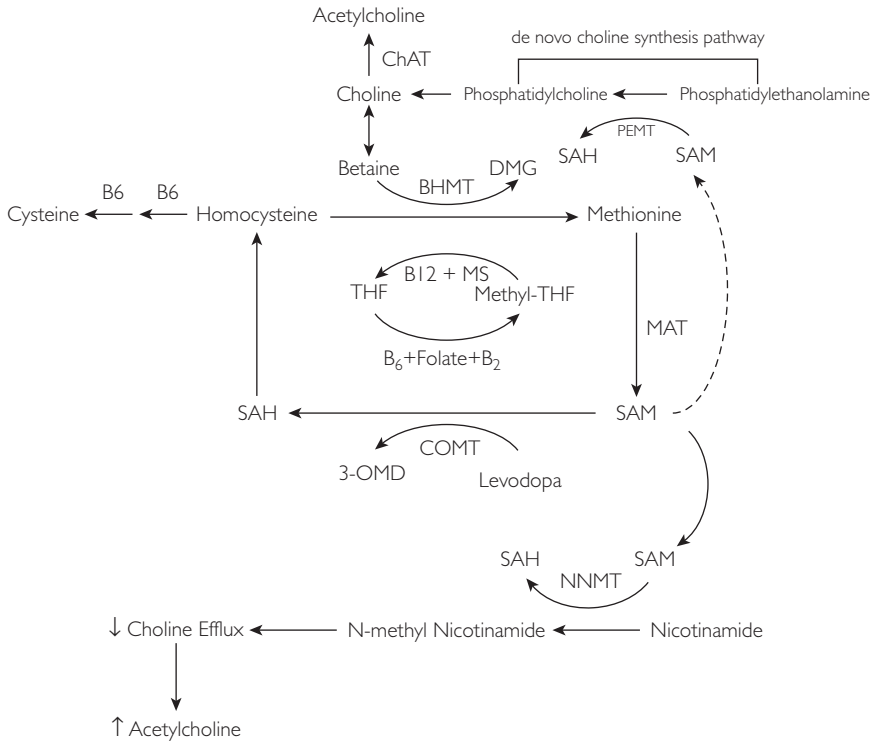

FIGURE. The relationship between vitamin B12 and acetylcholine through the transmethylation cycle. In normal physiologic states, homocysteine metabolism occurs through methylation (via MS and BHMT, which use B12 and choline, respectively) and transsulfuration (via vitamin B6). Vitamin B12 is also associated with *de novo* choline synthesis via the production of SAM, which is utilized by PEMT in the synthesis of choline from phosphatidylethanolamine. Further, SAM donates methyl groups to NNMT to form N-methyl nicotinamide, which competitively inhibits the efflux of choline from the CSF, increasing CSF free choline levels and, ultimately, acetylcholine. As such, decreased vitamin B12 levels may lead to increased free choline efflux from the CNS (through decreased N-methyl nicotinamide production). Levodopa supplementation increases homocysteine levels, requiring increased vitamin B12 and choline to methylate higher amounts of homocysteine. Further, levodopa requires the use of SAM to form 3-OMD, essentially reducing the amount of SAM available for PEMT and NNMT, thereby reducing choline levels. As such, levodopa therapy may worsen co-existent cholinergic deficiency by increasing choline use in the methylation of higher homocysteine levels and reducing available SAM for other methylation reactions. Overall the combination of loss of cholinergic neurons with the neurodegeneration of the pedunculopontine nucleus and nucleus basalis of Meynert with reduction of free choline in by levodopa-induced hyperhomocysteinemia may contribute to increased falls, postural instability, and dementia seen with progression of Parkinson disease. BHMT = betaine-homocysteine methyltransferase; B6 = vitamin B6; ChAT = choline acetyltransferase; COMT = catechol-O-methyltransferase; DMG = dimethylglycine; MAT = methionine adenosyltransferase; MS = methionine synthase; NNMT = Nicotinamide N-methyltransferase; PEMT = Phosphatidylethanolamine N-methyltransferase; SAH = S-adenosyl homocysteine; SAM = S-adenosyl methionine; THF = tetrahydrofolate; 3-OMD = 3-O-methyldopa.

the form of betaine) to methylate homocysteine to methionine in a reaction catalyzed by betaine homocysteine methyltransferase.[25] Choline not only serves as a precursor of acetylcholine but also of betaine and phosphatidyl choline. In the setting of low vitamin B12 levels, methylation of homocysteine would preferentially use betaine as a methyl donator, decreasing the amount of choline available for conversion to acetylcholine. (Figure) Finally, SAM also plays a role in *de novo* synthesis of choline via methylation of phosphatidylethanolamine by phosphatidylethanolamine N-methyltransferase to phosphatidylcholine and ultimately choline.

Evidence for the relationship between acetylcholine and vitamin B12 regulated metabolism comes largely from animal data.[23,26-28] However, the strongest evidence for an association between vitamin B12 and choline availability is based on a human study utilizing chronic total parenteral nutrition.[29] The authors reported that patients with vitamin B12 deficiency (as measured by elevated methylmalonic acid levels) had significantly lower serum choline levels (which are similar to those in the CSF) than patients with normal methylmalonic acid levels, despite adequate folate levels, suggesting that vitamin B12 and choline are interrelated regardless of folate status.[29] In animal studies, methyl-B12 has been shown to increase biosynthesis of acetylcholine through increased activity of choline acetyltransferase. In narcoleptic dogs, intraventricular infusion of methyl-B12 and choline both induced cataplexy and increased rapid eye movement sleep, both of which are partially mediated by acetylcholine.[26,27] Further, there was no difference in acetylcholine levels or learning in rats who were fed a choline-rich diet alone vs a choline deficient diet with vitamin B12 supplementation, providing further evidence that B12 is related to the metabolism of choline and, ultimately, acetylcholine.[28] 5′10′-methylene tetrahydrofolate reductase deficient mice (leading to a functionally deficient methionine synthase enzyme, mimicking vitamin B12 deficiency) have lower forebrain levels of choline, acetylcholine, N-methylnicotinamide, and cognitive performance compared with both wild type mice and 5′10′-methylene tetrahydrofolate reductase-deficient mice that were supplemented with either folate or S-adenosylmethionine.[23] Cognitive impairment in these mice improved following supplementation with folate or S-adenosylmethionine. The findings of this study suggest that S-adenosylmethionine (as well as folate and vitamin B12) decreases choline efflux by N-methylnicotinamide-mediated competitive inhibition of choline transporters resulting in increased acetylcholine levels through increased availability of free choline.[23]

Levodopa increases homocysteine production through the transmethylation cycle (Figure).[7] Levodopa-treated patients with PD have significantly higher homocysteine levels compared with both controls and levodopa-naïve patients with PD.[7] This occurs because the conversion of levodopa to 3-O-methyldopa requires methyl donation from S-adenosylmethionine, using the enzyme catechol-O-methyltransferase (COMT). This results in the increased production of S-adenosyl homocysteine, which is rapidly hydrolyzed to form homocysteine, leading to elevated levels of homocysteine.[7] High levels of S-adenosylhomocysteine inhibit SAM-mediated methylation reaction, including the formation of N-methylnicotinamide. Decreased N-methylnicotinamide then results in increased efflux of choline from the CNS. As levodopa leads to increased formation of S-adenosylhomocysteine (leading to subsequently decreased SAM reactions), levodopa therapy could theoretically lead to further choline efflux out of the CNS at higher doses and potentially worsen cholinergic dysfunction, particularly later in the disease course as cholinergic denervation continues; however, this remains a theoretical point and requires further study.

Vitamin B12 is most commonly measured as a total serum level. However, this method lacks sensitivity, making this a potentially insufficient measurement of true B12 status. Elevated homocysteine may also be used to distinguish subclinical B12 deficiency but is not specific to vitamin B12 status, as folate, vitamin B6, and betaine are intimately tied to homocysteine levels as well.[25] Serum methylmalonic acid is a more specific functional measure of B12 status. Methylmalonic acid levels increase in the setting of even mild vitamin B12 deficiency and are often elevated in patients still having serum vitamin B12 levels in the normal reference range.[30] Holotranscobalamin, the percentage of bound B12 to transcobalamin, may be an even more sensitive alternative to serum B12 measures, but this test is not widely available.[4] Importantly, concentrations of holotranscobalamin in the CSF have been reported to be 20% to 30% of that in plasma.[31] Further, CSF MMA levels are increased compared with serum, with a ratio of 2.65 in healthy controls, which

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increases to 8 in patients with neuropsychiatric symptoms due to vitamin B12 deficiency.[32] This suggests that alterations in CSF MMA may be a reflection of neuronal B12 status. Therefore, it is possible that, in CSF, vitamin B12 becomes depleted at a relatively early stage of deficiency that is not yet reflected by total serum B12, resulting in a functional CNS B12 deficiency, while serum B12 remains within a normal laboratory reference range.

Vitamin B12 and PD: A Potential Therapeutic Role?

Vitamin B12 levels in patients with PD have been demonstrated to be lower than controls.[3] Lower vitamin B12 levels are also associated with higher Hoehn-Yahr stage, cognitive impairment, and neuropathy in patients with PD.[33-35] Low vitamin B12 levels also correlate with more rapidly worsening ambulatory capacity in patients with PD.[4] In addition, low vitamin B12 and folate and high homocysteine concentrations have been shown to be independently associated with PD dementia compared with nondemented patients with PD, and higher homocysteine levels are associated with significantly worsening minimental status examination scores over time in patients with early PD.[4,36] It is clinically relevant to note that mean "lower" vitamin B12 levels in these cohorts still fall within the reference range for serum vitamin B12, and even patients with "normal" vitamin B12 levels have faster motor progression if in the low-normal quartile.[4] This, in part, reflects the poor sensitivity of the serum vitamin B12 measure but also suggests that in a disease state (such as PD) vitamin B12 needs may be higher, or CSF levels of vitamin B12 may become deficient even with low-normal serum vitamin B12 levels. Importantly, vitamin B12 levels do not appear to be associated with risk of developing PD.[3] An important point to consider is that vitamin B12 deficiency can cause subacute combined deficiency, cognitive impairment due to white-matter disease, and peripheral neuropathy, which could certainly contribute to cognitive and gait impairment in PD; however, is by no means conclusive in patients with PD, and more evidence is needed.[37] Finally, although vitamin B12 appears to be related to acetylcholine through multiple mechanisms detailed above, given the multiple physiologic processes the transmethylation cycle is involved in throughout the CNS, vitamin B12 deficiency causing cholinergic dysfunction is unlikely to be a straightforward linear causal process. However, given the possible association between the two, as well as low-risk supplementation with vitamin B12, this relationship deserves further exploration.

Conclusions

Lower levels of vitamin B12 have been associated with more rapidly decreasing ambulatory capacity, higher Hoehn-Yahr score, and cognitive impairment in PD. The cause of this relationship is unknown but is likely multifactorial. Cholinergic dysfunction has been associated with cognitive impairment and increased postural instability in PD. Vitamin B12 and acetylcholine metabolism are related through the SAM methylation cycle, and it is possible that this association may play a role in worsened PD symptoms in the setting of low vitamin B12 levels that has not previously been appreciated. Given the potential association between vitamin B12 and acetylcholine described above, and the at least partial role of acetylcholine in cognition and postural stability, further studies directly assessing the relationships among vitamin B12, acetylcholine, postural instability, and cognitive impairment (paying particular attention to confounders such as peripheral neuropathy) deserves further consideration. The heterogeneous neuroanatomic, neurochemical, and neurophysiologic nature of these symptoms in PD remain important to keep in mind, as acetylcholine is simply a small piece of a complex puzzle. Results of these studies could guide future clinical trials of high-dose vitamin B12 supplementation as a well-tolerated symptomatic adjunctive therapy for posture and gait instability and cognitive impairment in PD.

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