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Role of Vitamin B12 in the Synthesis of Iron-Sulphur Proteins

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ABSTRACT

Production of iron-sulphur proteins involves the movement of the sulphur atom from methionine into the sulphation pathway, via the enzyme cystathionine beta synthase. The activity of cystathionine beta synthase requires stimulation by high levels of S-Adenosylmethionine. Maintenance of S-Adenosylmethionine levels is dependent upon the methylation cycle, and more specifically of methyl-Co(III)cobalamin. Urinary Organic Acids Test data has been used to compare markers of functional vitamin B12 deficiency, with levels of the functional markers of aconitase deficiency (citrate) and succinate dehydrogenase deficiency (succinate). Studies reported here have shown for the first time that the activity of two iron-sulphur proteins, aconitase and succinate dehydrogenase are dramatically reduced in functional vitamin B12 deficiency. Such reduction is accompanied by increased secretion of succinic acid, aconitic acid and pyroglutamate. These studies have profound implications for understanding the processes involved in the development of diseases such as dementia, Parkinson's Disease, and autism, each of which is associated with the vitamin B12 deficiency marker, homocysteine.

Keywords

Aconitase, Iron-sulphur proteins, Pyroglutamate, Succinate dehydrogenase, Vitamin B12.

Introduction

There are two main active forms of vitamin B12, Adenosylcobalamin, an essential co-factor for the enzyme malonyl-CA-mutase, and methylcobalamin, an essential co-factor for the enzyme methionine synthase. Methionine synthase's only function is in maintenance of the methylation cycle, through the processing of homocysteine and 5-methyltetrahydrofolate, with eventual production of S-Adenosylmethionine (SAM), the universal methylating agent. Functional deficiency in vitamin B12 is generally assessed by an increase in methylmalonic acid (MMA), indicating a deficiency in Adenosylcobalamin and an increase in homocysteine, indicating a deficiency in methylcobalamin. Vitamin B12 deficiency is associated with many psychiatric symptoms such as apathy, agitation, impaired concentration, depression and insomnia [1-3]. Functional vitamin B12 deficiency has also been associated with chronic fatigue, which has been assumed to be due to lower production of the methylation product, creatine [3]. It has also been associated with developmental delay [4].

Apart from its direct role in methylation, however, increased methylation indirectly affects the sulphation pathway. Thus, increased levels of SAM stimulate the activity of the enzyme cystathionine beta synthase (CBS), a P5P dependent enzyme that catalyses the reaction L-Serine + L-homocysteine <=> L-cystathionine + H_2O. Hence movement of dietary sulphur into the sulphation pathway requires sufficient production of SAM (Figure 1). The sulphur in cystathionine is then used in the production of Hydrogen sulphide (H2S), Cysteine, with the subsequent production of glutathione, as well as in the formation of iron-sulphur complexes for iron sulphur proteins such as aconitase, succinate dehydrogenase, lipoate synthase and GABA amino-transferase. Reduced levels of cysteine, are accompanied by increased production of pyroglutamic acid. Comparison of neurotypical children with those with autism has shown that children with autism had lower serum and brain vitamin B12 [5], lower methionine, lower SAM:SAH ratio, higher serum homocysteine, and lower cystathionine, cysteine and glutathione, and lower aconitase activity than neurotypical children [6,7]. Reduced activity of CBS has been associated with mental retardation [8], whilst reduced aconitase activity has been associated with a lower mini mental score estimation mild cognitive impairment and

Alzheimer's diseases [9]. The current study has examined the relationship between a deficiency of Methyl B12 by the reduced activity of the Krebs cycle enzymes aconitase, and succinate dehydrogenase, and an increased level of pyroglutamate.

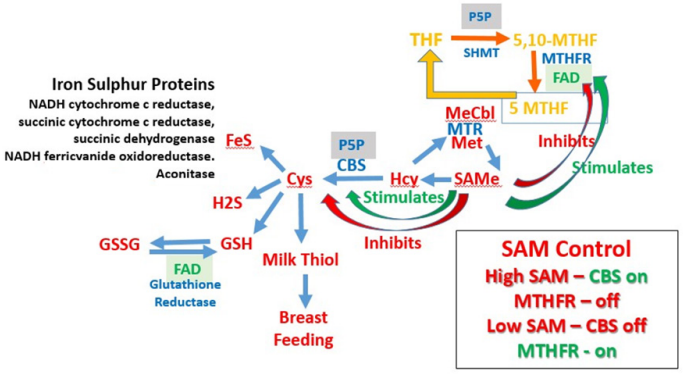

Figure 1: Importance of Methyl cobalamin (MeCbl) in maintenance of the methylation cycle and production of S-Adenosylmethionine (SAM).

Experimental Procedures

A retrospective analysis was performed upon data submitted to us for analysis from a cohort of 1750 children and adults from countries including Canada, USA, United Kingdom, Ireland, Bulgaria, France, Italy, India, Germany, Spain, Sweden, Serbia, Dubai, Croatia, New Zealand and Australia. No selection was made in the acceptance of data, with no data being rejected. Data is presented regardless of sex, or age. Ages varied from 1-year-old to seventy-four years old. Organic Acid Test Data (1750 sets, Mosaic Labs (formerly Great Plains Laboratories, Lenexa, KS, USA), which had been submitted to us for interpretation, from a variety of groups including parents of children with autism spectrum disorder, individuals with chronic fatigue syndrome and from persons who were healthy, and who had no previously identified health condition. Individual data is plotted as Scattergrams (see Figures 4 to 8). Data is presented as mmol/mol creatinine. Data was collected as per guidelines set out in the Declaration of the Helsinki principles.

Data analysis was carried out under the Australian National Health and Medical Research Council guidelines (NHMRC). Under these guidelines, all data was deidentified and steps were taken to ensure the anonymity and confidentiality of the data. Deidentification has consisted of absolute anonymity and confidentiality of the data, such that no specifics such as gender, ethnicity, Country of Origin, etc., is associated with any data point in the study. As such per the NHMRC guidelines.

Standard analysis of Adenosylcobalamin deficiency was calculated from MMA levels, whilst functional methyl B12 deficiency can be measured in OAT by increased levels of the neurotransmitter metabolite, HVA. Lack of activity of aconitase and succinate dehydrogenase results in increased urinary excretion of citric acid and succinic acid respectively.

Results

Organic Acid Test analysis (OAT) data was cross-correlated between adenosyl vitamin B12 insufficiency (MMA), Methyl vitamin B12 insufficiency, Homovanillic Acid (HVA), Methyl vitamin B12 insufficiency CoQ10 (3-hydroxyglutaric acid), and glutathione insufficiency, Pyroglutamic acid. In addition, a comparison was made between HVA and the activity of the two iron-sulphur proteins, aconitase (succinate) and succinate dehydrogenase (succinic acid). Correlation graphs for each are plotted below.

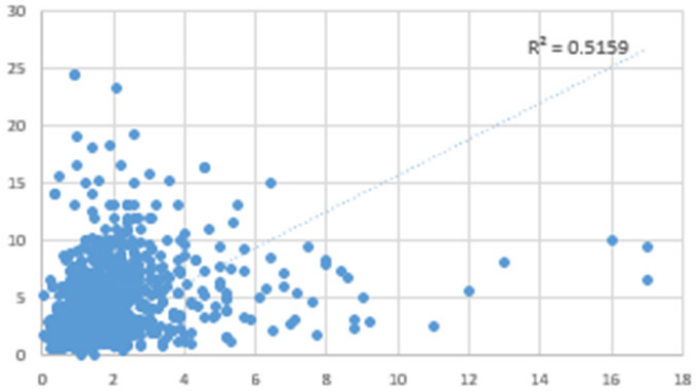

Figure 2: Relationship between HVA (horizontal axis) and MMA (vertical axis).

MMA is one of the standard markers of Adenosyl B12 deficiency. In absolute B12 deficiency the correlation between MMA (marker of Adenosyl B12 deficiency) is loosely correlated to markers of Methyl B12 deficiency such as HVA, VMA, QA, KA, HMG and Pyroglutamate. This correlation, is influenced by genetic variations in the methylation associated enzymes methionine synthase, methionine synthase reduction, methylenetetrahydrofolate reductase and serine hydroxymethyl transferase.

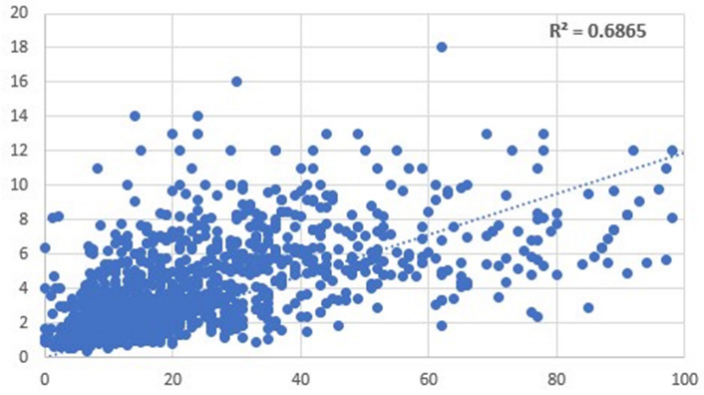

Figure 3: Relationship between HVA (vertical axis) and HMG (horizontal axis).

Formation of CoQ10 requires 3 methylation reactions. In functional methyl B12 deficiency, levels of the CoQ10 precursor HMG are

elevated, and energy transfer along the electron transport chain is reduced. As can be seen from the scattergram, the levels of the B12 deficiency marker, HVA correlated with those of the CoQ10 deficiency marker, HMG.

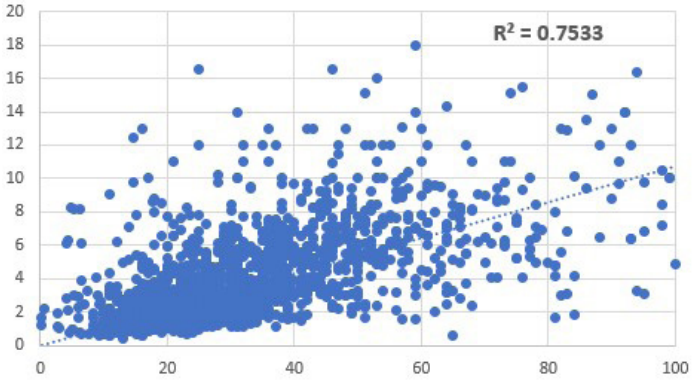

Figure 4: Reduced production of GSH in methyl B12 deficiency. Methyl B12 deficiency, as determined from increased urinary HVA (vertical axis) correlated with an increase in pyroglutamic acid (horizontal axis).

Production of glutathione requires the conjugation of glutamate, cysteine and glycine. In methyl B12 deficiency, the lower activity of the enzyme CBS results in reduced transfer of the sulphur in homocysteine into the sulphation cycle, with a resultant reduction in the levels of cysteine. In the absence of cysteine, the molecule, glutathione (GluCysGly) cannot form and pyroglutamate is formed. Methyl B12 deficiency as determined by increased HVA (vertical axis) was correlated with a marked increase in levels of urinary pyroglutamate (horizontal axis).

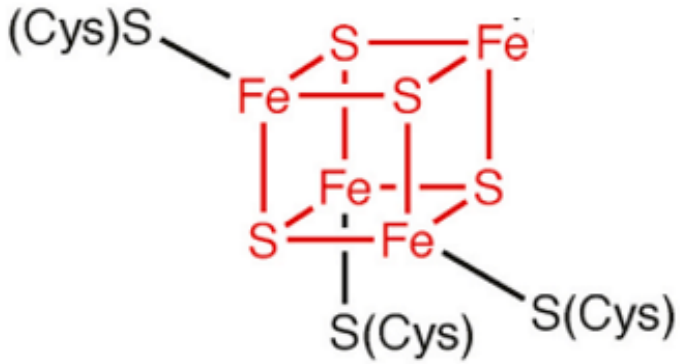

Figure 5: Iron-sulphur complex from aconitase, a [4Fe-4S] cluster.

Within the mitochondrial energy system, there are several iron-sulphur proteins, such as Complex I, Complex II and Complex III of the electron transport chain, and aconitase within the Krebs cycle [10]. The formation and activity of Iron-sulphur complexes is dependent upon the production of free-iron atoms, the presence of cysteine, and the formation of free Sulphur, generated in the sulphation pathway. A deficiency in iron, and/or reduced activity of the sulphation pathway results in reduced formation of iron-sulphur proteins and a reduced activity of the iron-sulphur proteins. Such reduction in activity is found when ferritin levels drop below 60 ug/litre where in the reduced activity of the iron-sulphur protein, aconitase, results in increased secretion of citrate (citric acid) into urine. Similarly, reduced methylation and reduced movement of methionine-derived sulphur into the sulphation pathway would also reduce the production of iron-sulphur complexes (See Figure 1). As would be expected an increasing level of Methyl B12 deficiency (as represented by increasing HVA), resulted in reduced activity of aconitase, and succinate dehydrogenase, with a resultant increase in citrate and succinate, respectively (Figure 6 and 7).

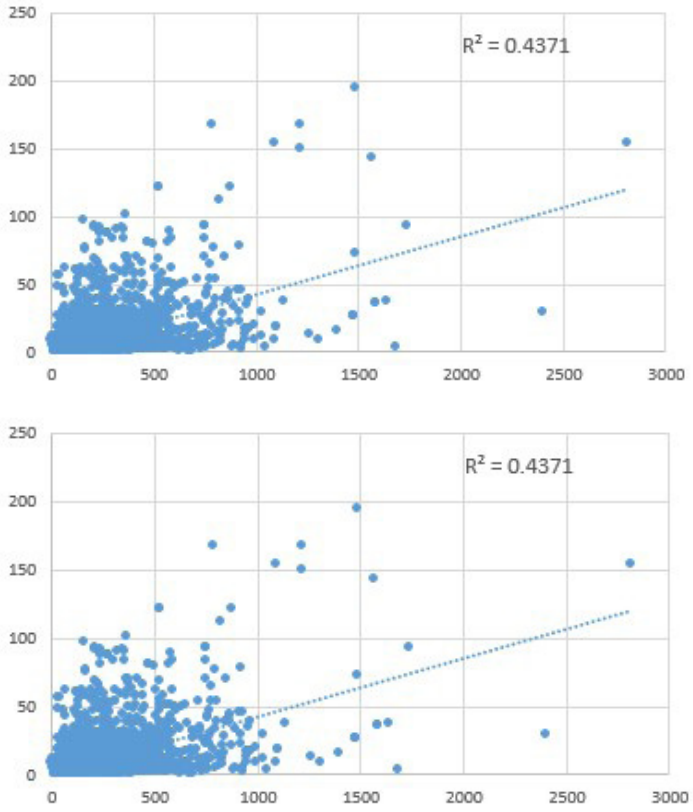

Figure 6: Relationship between HVA (vertical axis) and citrate (horizontal axis) (top panel), and HVA (vertical axis) and succinate (horizontal axis) (Bottom panel).

The activity of two iron sulphur proteins (aconitase – Figure 6. upper panel), and succinate dehydrogenase (succinate – Figure 6. Bottom panel) was related to the methyl B12 deficiency marker, HVA.

There was a rough correlation between loss of activity of two iron sulphur proteins aconitase and succinate dehydrogenase as determined by increases in the urinary levels of succinate and citrate.

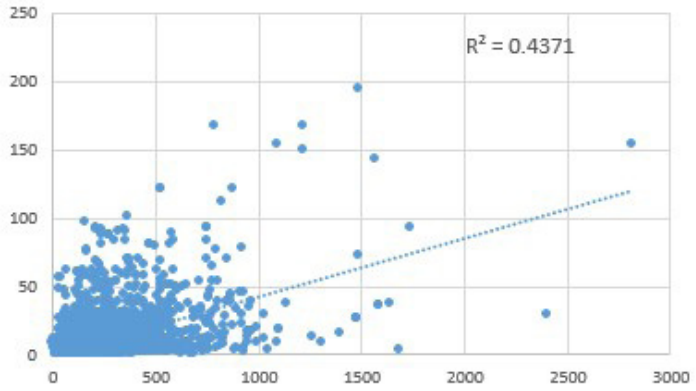

Figure 7: Relationship between succinate (vertical axis) and citrate (horizontal axis).

Discussion

Lower levels of vitamin B12 have been found in the frontal cortex of those with autism, with reduced activity of methionine synthase, with reduced levels of methylcobalamin, cystathionine, and glutathione, and higher levels of homocysteine, being observed [5,11].

Reduced aconitase activity has been found in those with Alzheimer's disease, mild cognitive impairment [9,12], as well as several neurodegenerative diseases [13], including ALS [14], Parkinsons disease [15], Friedreich's ataxia (FA) and Huntington's disease (HD) [16] and in the cerebellum and urine of those with autism [7]. The data presented suggests that lower levels of vitamin B12, which is common in conditions such as Alzheimer's disease, would result in decreased synthesis of the iron-sulphur proteins, such as aconitase and succinate dehydrogenase and the resultant energy loss may may well explain the lower cognitive function and the neurodegeneration observed in these conditions [17,18]. Low functional vitamin B12, particularly when combined with mutations in the cystathionine beta synthase gene would result in a further reduction in the production of iron-sulphur proteins, with resultant lower cognitive function. In this regard, mutations in the three key enzymes involved in homocysteine metabolism methylenetetrahydrofolate reductase [MTHFR], methionine synthase [MS], and cystathionine beta-synthase [CBS], have all been associated with an increased risk factor for Alzheimer's disease [19]. Similarly, increased vitamin B12 deficiency markers such as homocysteine, have also been associated with increased risk of AD [20-23].

Accompanying the decreased movement of dietary thiols into the sulphation pathway, such as occurs in functional B12 deficiency, there is also reduced production of glutathione, with an observed increase in the levels of pyroglutamic acid (Figure 4). Lower levels of reduced glutathione (GSH), have been found in Alzheimer's disease [24-29]. Defects in the sulphation pathway have previously been suggested as the cause for lower GSH levels, and supplementation studies using L-cysteine have resulted in partial restoration of GSH levels [30,31]. Data in the current study would support this hypothesis.

The results presented have application to the association of functional vitamin B12 deficiency, increased homocysteine, and the decline in cognitive function observed in the development of conditions such as Alzheimer's disease and autism. Hence, not only is there a reduced production of creatine, the primary product of the methylation cycle, but in functional vitamin B12 deficiency, there is an accompanying decline in the production of the CoQ10 energy shuttle vector of the electron transport chain [32] (Figure 3), and in addition there is a decline in the activity of the two important iron-sulphur enzymes, aconitase and succinate dehydrogenase (Figures 6a, 6b, 7), with an accompanying energy loss from the Krebs cycle, and lack of energy transfer from the Krebs cycle to the electron transport chain, with resultant excretion of citrate and succinic acid into urine. Presumably, there would be a reduced energy transfer due to reduced activity of the iron-sulphur proteins that comprise complex II and III of the ETC. This loss of energy would also present as "frailty" and "fatigue" in conditions such as Alzheimer's disease, and may also explain the weight loss that is common Alzheimer's disease and prolonged vitamin B12 deficiency [33-37].

Summary

Assessment of urinary organic acids from over 1700 people has shown that there is a correlation between markers of increasing vitamin B12 deficiency with levels of HMG, reflective of reduced production of the methylation product, CoQ10 and pyroglutamate, being indicative of reduced production of reduced glutathione. The data presented is the first example of the effect of methyl B12 deficiency, being shown to affect the production and/or activity of the iron-sulphur proteins, aconitase and succinate dehydrogenase, which is indicative of a reduced activity of the sulphation pathway. The observed reductions have profound implications for the reduction in mental capacity observed in conditions such as autism and Alzheimer's disease.

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