Physicochemical Characteristics, Biological Functions and Deficiency of Cobalamins

Saima Zahid, Kiran Qadeer and Iqbal Ahmad

ABSTRACT
Cobalamins are complex organometallic compounds of biological interest present in animal food products including meat, eggs and milk. The clinically important cobalamins, i.e. cyanocobalamin (vitamin B12), hydroxocobalamin and methylcobalamin exist as coenzymes in biological system and are involved in several biochemical functions. The deficiency of vitamin B12 occurs mainly due to its malabsorption and results in pernicious anemia. This disease is age related and is associated with the destruction of parietal cells in the stomach. Clinical studies with high dose supplementation of cobalamins suggest a strong correlation between the duration of cognitive symptoms of the disease and the therapy. Neural tube defects and other birth defects have also been reported with cobalamin deficiency.

INTRODUCTION
Vitamin B12 and its derivatives remain the center of interest for pharmacists and chemists because of being an extraordinary group member of organometallic complexes. They are classified as cobalamins, a class of octahedral Co(III) complexes. Vitamin B12 was first discovered in 1948, later named as cyanocobalamin3 and was first synthesized in 19734. Cobalamins are exclusively synthesized by bacteria and primarily found in animal products5,6. The richest sources of cobalamins are animal organs (specially liver and kidney) and meat7. These are found in those tissues which require the vitamin for critical function in cellular division and growth. Some animal tissues have the ability to store the vitamin in such amounts which are sufficient enough to meet the requirements of the organisms for long periods of deprivation. Fish, shell fish, mushrooms, eggs, milk and bacterially fermented foods are enriched with vitamin B12. Plant derived foods are devoid of B12 unless they are contaminated6,7.
Some algae also contain cobalamins but such analogues are ineffective7 such as edible cyanobacteria (Spirulina, Apahhizomenon, Nostoc) and are often cited as vitamin B12. However, they are enriched with large amounts of pseudovitamin B12 (7-adeninyl cyanocobamide) which is biologically inactive and may antagonize the utilization of vitamin B126,8.
The principal vitamer cobalamins in food are methylcobalamin, adenosylcobalamin and hydroxocobalamin. Methylcobalamin and hydroxocobalamin are the dominant forms of cobalamins that are present in dairy food items while adenosylcobalamin and hydroxocobalamin are present in meat9.

CHEMICAL STRUCTURE
Cobalamins possess a complex structure based on corrin ring system. Four reduced pyrrole rings are linked together to form a corrin nucleus which is chelated by four pyrrole nitrogens. All compounds that contain such a ring system are termed corrinoids. This macrocyclic ring is coordinated centrally to a cobalt atom (Fig.1).
The molecule can be divided into two halves, a planar group and a nucleotide which are set at right angle to each other. Corrin ring constitutes the planar group while nucleotide consist of dimethylbenzimidazole and a phosphorylated sugar (ribose 3-phosphate). The fifth ligand of the cobalt may be occupied covalently by different anionic groups giving rise to various forms of cobalamins. The ligand of the anionic group (X) can be one of the following types.

- CN – cyanocobalamin
- OH – hydroxocobalamin
- H2O – aquacobalamin
- NO2 – nitritocobalamin
- SO3 – sulfitocobalamin
- CH3 – methylcobalamin
- 5-deoxyadenosyl – adenosylcobalamin

In cyanocobalamin and the naturally occurring hydroxocobalamin forms the cobalt atom is trivalent Cob(III)amin, the most oxidized form.

**PHYSICOCHEMICAL CHARACTERISTICS**
The physicochemical characteristics of clinically important cobalamins are given in Table 1.

**ABSORPTION, TRANSPORTATION AND METABOLISM OF VITAMIN B12**
Vitamin B12 is present in protein bounded forms in foods. Dietary B12 is released from the protein complex in the acidic environment of stomach by the action of pepsin. The released B12 then binds to salivary R binders. This complex is then hydrolyzed by proteases in the small intestine. The free B12 is available to bind with the intrinsic factor (gastric glycoprotein). The IFB12 complex then binds to IF receptor. All forms of vitamin B12 are absorbed by IF dependent mechanism. The passive diffusion is another mean of B12 absorption occurring equally through the absorption surface of gastrointestinal tract. Approximately 1-2% of the oral dose of vitamin B12 is absorbed by this mechanism.

**BIOLICAL FUNCTIONS OF COBALAMINS**

**Metabolic Function**
Cobalamins are required by cellular enzyme, as cofactor in either the adenosylcobalamin or the methylcobalamin form. Methylcobalamin is required for the remethylation of homocysteine to methionine catalyzed by methionine synthase. Methionine synthases are large monomeric zinc metalloproteins and comprise of the three domains, a catalytic domain which contains the binding site for methyltetrahydrofolate and homocysteine, a B12 domain where B12 cofactor binds and an accessory protein domain. In this reaction, methyltetrahydrofolate is involved as an intermediate acceptor of the methyl group. The cob(I)amin cofactor is methylated by 5-methyltetrahydrofolate generating enzyme bound methylcob(III)amin and releasing tetrahydrofolate. The methyl group is transferred by methylcob(III)amin to homocysteine to generate methionine and regenerate the enzyme bound cob(I)amin cofactor. Occasionally this cofactor is oxidized to the non-functional cob(II)amin form during catalysis. The enzyme is reactivated by methionine synthase reductase that catalyzes the adenosylmethionine and NADPH dependent reductive methylation of enzyme bound cob(II)amin to methylcob(III)amin. In all cobalamin deficiency states methionine synthesis is impaired and homocysteine can accumulate.

Further the methionine synthesis reaction provides tetrahydrofolic acid (THF), which is an essential form for other folate dependent reactions. The adenosylcoabamin present in mitochondria is required as a coenzyme for methyl-malonyl – Co A mutase. This enzyme catalyzes the conversion of methylmalonyl-Co A to succinyl-Co-A21 which then enters to Kreb’s cycle.

**Other Functions**
Cobalamins may function to modulate the oxidative stress responses, including inflammatory response. Inflammatory diseases are associated with increased blood levels of transcobalamin. Cobalamins may function as antioxidant which could result from combination of direct or indirect effects of stimulation.
methionine synthase activity\textsuperscript{22,24}. Direct reaction with reactive oxygen and nitrogen species, a glutathione sparing effects\textsuperscript{25} and modification of signaling molecule\textsuperscript{26} result in induction of stress responses. Thiolutocobalamin in particular functions that is; in one or more of these potential mechanisms exhibiting a marked antioxidant activity and significant cellular protection against oxidative stress\textsuperscript{23}. The B12 derivatives also possess antimalarial activity. Adenosylcobalamin, methylcobalamin and aquacobalamin are approximately forty times more potent inhibitor of β-haematin formation than chloroquine. The suggested mechanism of interaction is the inhibition of β-haematin formation by β–interaction of their corrin ring with the Fe(III)protoporphyrin ring and by hydrogen bonding using their \textsuperscript{5,6} dimethylbenzimidazoyl ring or ribose moiety or sugar side chain but they are weak antimalarial than chloroquin or quinine\textsuperscript{27}.

**DEFICIENCY OF VITAMIN B12**

The deficiency of vitamin B12 occurs mainly due to its malabsorption and rarely due to dietary insufficiency. The classical manifestation is pernicious anemia, which is a megaloblastic anemia, and occurs from the inability to absorb vitamin B12 due to lack of intrinsic factor. This disease is age related and usually associated with the destruction of parietal cells in the stomach. It is an autoimmune disease in which antibodies to the H-adenosine triphosphatase (ATPase) or to IF are produced. The malabsorption of dietary B12 can also be associated with any condition of decreased acid production or pancreatic insufficiency. Helicobacter pylori infection may lead to atrophic gastritis which in turn results in impairment of B12 absorption because of the inability to release the vitamin from protein binders, while absorption of B12 in the absence of food is unimpaired\textsuperscript{10}.

The B12 associated pernicious anemia is sometime identical to megaloblastic anemia observed with folate deficiency. In B12 deficiency, the B12 dependent methionine synthase enzyme is inactive and cytosolic folate is trapped as 5-methyl tetrahydrofolate causing deficiency of folate which is required by other folate coenzyme forms such as thymidylate synthesis, leading to functional folate deficiency in the cell. Defective DNA is synthesized as a result of this induced secondary folate deficiency, high levels of folate supplementation can cause a hematological response in megaloblastic anemia associated with B12 deficiency but it is ineffective to correct neurological pathologies occurring due to its deficiency\textsuperscript{10}. Many patients with B12 deficiency have neither anemia nor macrocytosis\textsuperscript{28,29,30,31,32,33} therefore, the determination of B12 and folate levels are recommended in all anemic patients or patients with macrocytosis or neurologic or psychiatric symptoms. B12 deficiency and hyperhomocysteinemia are considered as risk factors for stroke and ischemic heart disease, patients with normal levels may have cellular deficiency\textsuperscript{29,34}. Vitamin B12 deficiency is the most common problem in elderly people as the cobalamin absorption is often decreased\textsuperscript{35,36} and can increase the risk of atherosclerosis\textsuperscript{37,38,39} and neurodegenerative disease\textsuperscript{40,41}. Insufficient cobalamin levels, or increased levels of homocysteine or methylmalonic acid concentrations are also associated with cognitive impairment\textsuperscript{7,36}.

Mood swings are also reported with B12 deficiency, depression is most common in B12 deficient subjects\textsuperscript{7,42}. Clinical studies with high dose supplementation of cobalamins suggested a strong correlation between duration of cognitive symptoms and response to therapy\textsuperscript{43}. The exact reason is not known to date but experimental findings suggested a relation between metabolites Sadenosylmethioine (SAM) and homocysteine. SAM serves as methyl group donor in neurophysiologically relevant reactions\textsuperscript{44}, such as, methylation of phospholipids and synthesis of different neurotransmitters (e.g. acetylcholine). The demethylation of SAM results in Sadenosylhomocysteine (SAH) which acts as an inhibitor of SAM dependent transmethylation reactions\textsuperscript{40,41,45}. In B12 and folate deficiency states the remethylation of homocysteine to methionine is inhibited resulting in less methionine availability for
SAM synthesis. The hypomethylation is one of the factor responsible in various neurological and psychiatric disease such as dementia and depression\textsuperscript{46}. Neurodegeneration (mostly of glial cells, myelin and interstitium) also occur due to B12 deficiency\textsuperscript{6}. Low B12 status may exacerbate multiple sclerosis by enhancing the processes of inflammation and demyelination, and by impairment of myelin repair\textsuperscript{6}. Neural tube defects and other birth defects are also reported with cobalamin deficiency. Bone health and hearing ability\textsuperscript{48} is also effected by B12 levels. B12 supplementation lessens the tinnitus and osteoporosis consequences.

CONCLUSION
Vitamin B12 and its clinically useful derivatives belong to a group of organometallic complexes which were discovered as early as 1948. The principal vitamer cobalamins present in food are methylcobalamin, adenosylcobalamin, hydroxocobalamin and cyanocobalamin. The deficiency of vitamin B12 results in pernicious anemia due to its malabsorption because of inadequate production of IF by gastric parietal cells, and/or defective functioning of ileal IF-receptors. The cobalamins may function to modulate the oxidative stress responses including inflammatory response. The inflammatory diseases are associated with increased blood levels of transcobalamin. Cobalamains may also function as antioxidant and react with reactive oxygen and nitrogen species. The B12 derivatives also possess antimalarial activity. Vitamin B12 deficiency is most common in elderly people due to malabsorption of vitamin.

REFERENCES


