Cobalamins and Methylcobalamin: Coenzyme of Vitamin B12

Şeyda AKKUŞ ARSLAN¹, İsmail ARSLAN², Figen TIRNAKSIZ³

SUMMARY

Vitamin B12, also called cobalamin, is one of the B vitamins. Cobalamin may refer to several chemical forms of vitamin B12, depending on the ligand of the cobalt ion. These are cyanocobalamin, hydroxycobalamin, methylcobalamin and adenosylcobalamin. For many years preparations of vitamin B12 (cobalamin) have been finding use in medicine. All of the forms of cobalamin are present in substantial amounts in the human and animal organism. Cobalamin plays a specific role in amino acid metabolism, i.e. in the methionine synthase reaction. Crystalline cobalamins are administered parenterally (intramuscularly) or orally (except hydroxycobalamin) for treating deficiency states. The absorption of physiological doses of cobalamin is limited to approximately 1.5 – 2 μg/dose. Methylcobalamin is one of the two coenzyme forms of cobalamin. It is the metabolically active form required for cobalamin-dependent enzyme function. Intramuscular administration is widely accepted as a treatment method. Oral cobalamin supplementation is also used but it is considered to be less reliable. Therefore, a nanoemulsion formulation was developed to overcome this problem by the authors. In this review, especially the general information about cobalamin and methylcobalamin will be shared. Besides, information about the developed nanoemulsion will be given.

Key Words: Cobalamin, methylcobalamin, vitamin B12.

ÖZET


Anahtar kelimeler: Kobalamin, metilkobalamin, vitamin B12.
INTRODUCTION

Cobalamins

Vitamin B12 is the generic name given to a group of related compounds containing cobalt as the central ion in a corrin ring (Figure 1). This group of biologically active cobalt containing corrinoids is also described as ‘cobalamins’. The cobalt ion can be coordinated to a methyl-, 5’-deoxyadenosyl-, hydroxy- or cyano- group (Wolters et al., 2004). Hydroxycobalamin and cyanocobalamin used in food supplements are transformed in the human body by coordinating with other ligands into methylcobalamin and 5’-deoxyadenosylcobalamin. The methylcobalamin and 5’-deoxyadenosylcobalamin are actively involved in endogenous metabolism (Hillman, 1985; Kelly, 1997).

Cobalamin plays a specific role in amino acid metabolism, i.e. in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA into succinyl CoA. In humans these cobalamin dependent reactions have been identified with methionine synthase functioning with methylcobalamin and the methylmalonyl coenzymeA mutase reaction with 5’-deoxyadenosylcobalamin as the active coenzyme (Aguilar, 2008). It is considered to function as an essential factor in DNA synthesis for chromosomal replication and division (Kaushansky and Kipps, 2011).

Intestinal uptake of cobalamin occurs in the terminal ileum (Ermens et al., 2003). Cobalamin is absorbed and transported across cellular plasma membranes by two mechanisms, including (Castle and Hale, 1998; Andre’s et al., 2004):

1) Active transport. Endocytosis of dietary cobalamin bound to gastric intrinsic factor (IF) by ileal enterocytes, which is a receptor-mediated process with a specific IF-cobalamin receptor.

2) Passive diffusion. Significant amounts of the vitamin can be absorbed with this process when large quantities of cobalamin are ingested. The rate of absorption by the passive process has been reported to be 1% of the ingested amount of cobalamin (Scott, 1997; Baik and Russell, 1999).

It is considered that, dietary cobalamin is absorbed...
by the active transport mechanism. The commonest
disorder causing cobalamin deficiency in Europe and
North America is pernicious anemia in which IF is defici-
cent as a consequence of atrophy of the fundal mucosa.
In such patients, oral cobalamin replacement therapy is
ineffective (Lancaster, 1980).

It was concluded that the physiological dose of co-
balamin, that healthy individuals need, is approximately
1.5 – 2 μg/dose. Regardless of dose, approximately 1.2%
of cobalamin is absorbed by passive diffusion and con-
sequently this process becomes quantitatively important
at high levels of exposure (Aguilar et al., 2008). Cur-
rent intake recommendations are 2.4 μg/day for adults,
slightly more during pregnancy (2.6 μg/day) and lacta-
tion (2.8 μg/day) (Sloan, 2008).

All the cobalamins have the same pattern of adverse
reactions. The adverse effects of high doses of cobala-
mins include urticaria, eczematous and exanthematous
skin lesions, and anaphylactic reactions, but it is not
clear whether the reactions are caused by the drug it-
self, a preservative, or possibly by contaminants. High
oral or parenteral doses of vitamin B6 and especially
hydroxycobalamin are also on rare occasions suspected
to induce acne which is, however, always benign (Dupre
et al., 1975). Several cases of cobalamin-induced fol-
liculitis and acneiform eruptions have been described,
in one case in connection with a patient receiving to-
tal parenteral nutrition (Heyworth-Smith and Hogan,
2002). Adverse effects associated with the parenteral
administration of hydroxycobalamin include mild di-
arrhea, urticaria, skin rash, and anaphylactic reactions.
Cross-sensitivity of hydroxycobalamin and cyanoco-
balamin has been reported (Hathcock and Troendle,

Cobalamin is stored in the liver, 3 – 5 mg being
present in normal subjects. As the daily requirement
is very small (2 – 3 μg/day), symptoms of cobalamin
deficiency do not develop for 3 – 10 years after the
development of cobalamin malabsorption. Negligible
amounts of dietary cobalamin are excreted unchanged in
the urine, except when large amounts are administered
parenterally (Lancaster, 1980).

The main causes of cobalamin deficiency are perni-
cious anemia (20–30%) and food-cobalamin malab-
sorption (50–70%) in elderly (Andre’s et al., 2000).
Food-cobalamin malabsorption syndrome, which has
only recently been identified, is a disorder characterized
by the inability to release cobalamin from food or its
binding proteins (Dawson et al., 1988; Carmel, 1995).
The partial nature of this form of malabsorption might
produce a more slowly progressive depletion of cobala-
min than does the more complete malabsorption en-
gendered by disruption of intrinsic-factor-mediated ab-
sorption ( Andre’s et al., 2005). This syndrome is usu-
ally caused by atrophic gastritis, related or unrelated to Heli-
cobacter pylori infection, and long-term ingestion of ant-
acids (Bellou et al., 1996) and biguanides (Sorich et al.,
2008). In food-cobalamin malabsorption, the absorp-
tion of ‘unbound’ cobalamin (free crystalline) is normal
(Carmel, 1995). Second, between 1 and 5% of free
cobalamin (or crystalline cobalamin) is absorbed along
the entire intestine by passive diffusion. This absorption
explains the mechanism underlying oral treatment of
cobalamin deficiencies (Hathcock and Troendle, 1991;
Lane and Rojas-Fernandez, 2002; Andre’s et al., 2004).

The representative symptoms of cobalamin deficien-
cy are megaloblastic anemia and peripheral neuropathy,
reflecting the fact that the hematopoietic and nervous
systems are readily affected (Goto et al., 2015).

Classical Treatment of Cobalamin Deficiency

The classical treatment for cobalamin deficiency,
particularly when the cause is not dietary deficiency, is
parenteral administration because of the unpredictable
efficacy of oral treatment – in most countries intramus-
cular injection – of this vitamin (in the form of cyano-
cobalamin and, more rarely, hydroxy or methylcobala-
min) (Lane and Rojas-Fernandez, 2002; Andre’s et al.,
2004; Hvas and Nexo, 2006; Andre’s et al., 2007 ).

The medical treatment of cobalamin deficiency in-
volves regular, usually monthly, intramuscular (im) co-
balamin injections for life. However, traditions concern-
ing both dose and schedule of administration vary con-
siderably (Andre’s et al., 2005). In France, the treatment
involves the administration of 1000 μg of cyanocoba-
lin per day for 1 week, followed by 1000 μg/week for
1 month, followed by 1000 μg/month, normally for the
rest of the patient’s life (Andre’s et al., 2004). In USA
and UK, dosages ranging from 100 to 1000 μg/month
(or every 2–3 months when hydroxycobalamin is given)
are used during the rest of the patient’s life (Andre’s et
al., 2007).

Oral Therapy

As cobalamin is absorbed by IF-independent passive
diffusion, daily high dose (pharmacological dose) oral
cyano-cobalamin can induce and maintain remissions in
patients with megaloblastic anemia (Elia, 1998; Lane and Rojas-Fernandez, 2002). In cases of cobalamin deficiency other than those caused by nutritional deficiency, alternative routes of cobalamin administration have been used in a curative perspective: oral (Lane and Rojas-Fernandez, 2002; Solomon, 2007) and nasal (Slot et al., 1997; Vidal-Alaball et al., 2005). These other routes of administration have been proposed to prevent discomfort, inconvenience and cost of monthly injections (Andres et al., 2007). A recent review of Lane (Lane and Rojas-Fernandez, 2002) has reported preliminary data of the usefulness of oral cobalamin treatment. It is notable that to date, oral cobalamin curative treatment accounts for more than 70% of the total cobalamin prescribed in Sweden in 2000 (Van Asselt et al., 1998). Historically, the Swedish team was the first to routinely propose oral cobalamin therapy to cure cobalamin deficiency (Harthcock and Troendle, 1991).

### Methylcobalamin

The chemical name for methylcobalamin is Coα-[(α-(5,6-dimethylbenz-1H-imidazolyl))-Coβ-methylcobamide. Synonyms and trade names are meco- 
balamin, methylcobalamin, cobaltrimethylcobalamin, Algbaz and Cobamet. Its molecular weight is 1344.4 g/mol (Aguilar et al., 2008). Methylcobalamin is one of the two coenzyme forms of cobalamin (the other being adenosylcobalamin). They are the metabolically active forms required for cobalamin-dependent enzyme functions. Evidence indicates these coenzyme forms of cobalamin, in addition to having a theoretical advantage over other forms of cobalamin, actually do have metabolic and therapeutic applications not shared by the other forms of cobalamin. Methylcobalamin is a cofactor in the enzyme methionine synthase which functions to transfer methyl groups for the regeneration of methionine from homocysteine (Bachmann, 2016).

Methylcobalamin has been available in Japan since the mid-1970s, and is used in peripheral neuropathies, neuritides, polyneuropitides, and a number of movement disorders (Tsukerman et al., 1991). In France, methylcobalamin is used for treating pain syndromes of neurological origin. Positive results have been obtained using methylcobalamin to treat experimental diabetes, toxic hepatitis and hyper chronic anemia in rats and rabbits (Tsukerman et al., 1991).

A therapeutic dose for conditions requiring methylcobalamin would be a minimum of 1500 μg and a maximum of 6000 μg per day. No significant therapeutic advantage appears to occur from dosages exceeding this maximum dose; however, it is likely that beneficial physiological effects occur at dosages as low as 100 μg per day, especially if this dose is given repetitively over time (Bachmann, 2016). Clinical studies have reported no adverse effects following administration of up to 6.0 mg/day of methylcobalamin for several weeks and up to 1.0 mg/day cyanocobalamin for several years (Aguilar et al., 2008). Methylcobalamin have been administered orally, intramuscularly, and intravenously; however, positive clinical results have been reported irrespective of the method of administration. It is not clear whether any therapeutic advantage is gained from non-oral methods of administration (Bachmann, 2016).

Methylcobalamin have usually been administered in divided doses three times daily. These supplements have excellent tolerability and no known toxicity. No rationale exists to suspect methylcobalamin would not also be safe during pregnancy (Bachmann, 2016).

The quantity of methylcobalamin to be added to food supplements will be determined by individual formulators but is normally the quantity necessary to supply adults up to 500 μg cobalamin/day (Aguilar et al., 2008). Methylcobalamin is equivalent physiologically to cobalamin, and can be used to prevent or treat pathology arising from a lack of cobalamin (cobalamin deficiency), such as pernicious anemia.

Most of the cobalamin in blood and breast milk is methylcobalamin (Sloan, 2008). Evidence indicates cobalamin from methylcobalamin is utilized more efficiently than cyanocobalamin to increase the levels of coenzyme forms of cobalamin. Although free methylcobalamin is not very stable in the gastrointestinal tract, and considerable loss of the methyl group can take place under experimental conditions, in physiological situations if probably partially protects methylcobalamin from degradation. Paper chromatography of digested ileal mucosa has demonstrated unchanged absorption of methylcobalamin following oral administration. The quantity of cobalamin detected following a small oral dose of methylcobalamin is similar to the amount following administration of cyanocobalamin; but, significantly more cobalamin accumulates in liver tissue following administration of methylcobalamin. Human urinary excretion of methylcobalamin is about one-third that of a similar dose of cyanocobalamin, indicating substantially greater tissue retention (Bachmann, 2016).

Although it has been suggested that oral cobalamin treatment may be effective for food-cobalamin malabsorption, most of the internists believe that there were
no effective oral cobalamin preparations (Lederle, 1991; Herbert, 1996; Chalmers et al., 2000). A retrospective study was performed to reveal the lack of oral treatment in B12 deficiency by the authors of this review. The results of retrospective study showed that parenteral treatment was preferred for the patients who have vitamin B12 deficiency (Akkuş Arslan et al., 2016) (Table 1).

To overcome this problem, an oral nanoemulsion formulation of methylcobalamin was developed by the authors Akkuş Arslan, Arslan and Türnaksız (Akkuş Arslan et al., 2016). The aims of the study were to investigate if the developed nanoemulsion is effective as much as parenteral form and to compare bioavailability of developed nanoemulsion with commercial tablet and intramuscular forms of vitamin B12. The serum analysis results were given in Table 2.

Table 1. Investigation results of retrospective study

<table>
<thead>
<tr>
<th>Location</th>
<th>Ankara Educational Research Hospital, Department of Family Medicine, Polyclinics of Bahcelievler-Ulus-Yenimahalle-Hüseyingazi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of research</td>
<td>2012 and 2013</td>
</tr>
<tr>
<td>% im treatment</td>
<td>100</td>
</tr>
<tr>
<td>% oral treatment</td>
<td>0</td>
</tr>
<tr>
<td>Number of patients</td>
<td>592</td>
</tr>
</tbody>
</table>

Table 2. The results of analysis of the serum samples

<table>
<thead>
<tr>
<th>Dose (µg/kg)</th>
<th>t\textsubscript{max} (hour)</th>
<th>Administration way</th>
<th>Formulation type</th>
<th>Form of B12</th>
<th>Mean serum concentration* at t\textsubscript{max} (pg/mL) (n=3, X±SS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>2</td>
<td>Oral</td>
<td>Nanoemulsion</td>
<td>Methylcobalamin</td>
<td>4250 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intramuscular</td>
<td>Parenteral</td>
<td>Cyanocobalamin</td>
<td>4570 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral</td>
<td>Tablet</td>
<td>Cyanocobalamin</td>
<td>3950 ± 3</td>
</tr>
</tbody>
</table>

* The serums were analyzed by using ‘rat, vitamin B12, ELISA, Cusabio kits’ using 1/100 dilution.

According to the results; the use of active form of vitamin B12 (methylcobalamin) dramatically affected the serum levels, the nanoemulsion found more bioavailable in comparison with commercial tablet form, the bioavailability of developed nanoemulsion found closer to commercial parenteral form and a non-invasive and effective formulation could able to be developed with the study.

**CONCLUSION**

Cobalamin is an essential vitamin that has to be used in the patients who have cobalamin deficiency and for the individuals who use the cobalamin for daily requirement. Methylcobalamin is one of the two coenzyme forms of cobalamin utilized in the cobalamin-dependent enzymes in humans. It is a cofactor in the enzyme methionine synthase which functions to transfer methyl groups for the regeneration of methionine from homocysteine. It offers a theoretical advantage in cobalamin supplementation. Methylcobalamin is retained in the body better and increase tissue concentrations of cobalamin better than cyanocobalamin. Additionally, it demonstrates a range of activity and clinical results not shown by the other supplemental forms of cobalamin. The metabolic fate and biological distribution of methylcobalamin and 5′-deoxyadenosylcobalamin are expected to be similar to that of other sources of cobalamin in the diet. The use of 5′-deoxyadenosylcobalamin and methylcobalamin as a source of cobalamin in food supplements for the general population at the proposed uses and use levels is not of safety concern.

Most of the medical doctors prefer parenteral treatment for the patients who have cobalamin deficiency, because they do not believe in the efficiency of oral treatment. But oral cobalamin formulations were told to be effective and the results of our study confirm that an effective oral cobalamin nanoemulsion system could be developed.
REFERENCES


Sloan MA. Designing and Pilot Testing a Database to Properly Track Nutrient Consumption in Overweight, Postpartum Women and Infants Enrolled in a Dietary Intervention Study (Dissertation) Oxford (Ohio): Miami University, Department of Nutritional Sciences of the College of Allied Health Sciences. 2008.