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Complex B vitamins: Physiology and Therapeutic Effect on Pain

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Abstract Complex B vitamins (B1, B6, and B12) have shown affect several metabolic routes in the organism. Vitamin B1, or thiamine, has anti-oxidative properties and significant effects on the nitric oxide-cGMP pathway, giving it anti-hyperalgesic and anti-allodynic properties. Vitamin B6, or pyridoxine, has modulatory effects on calcium channels, inhibiting glutamate synthesis and intervening in the metabolism of carbohydrates. Vitamin B12 shows reconstructive effects and increases epidermal growth factor synthesis. Combining them produces an additive effect that, apart from participating in vital body functions, can help treat diseases such as pain. It is important to know about the mechanisms that explain their analgesic and anti-allodynic effects, in order to understand the physiologic mechanisms involved in these actions. This article summarizes Vitamins B1, B6 and B12 actions, focusing in pain treatment.

Keywords: *thiamine, cobalamine, pyridoxine, pain nitric oxide, oxidative*

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1. Introduction

Complex B is constituted by 8 vitamins, essential for cells to function, since they help the body to transform food into energy. [1] They are water-soluble and, therefore, they may get lost in cooking-water and, if taken excessively, eliminated in urine. These vitamins are B1 (thiamine); B2 (riboflavin); B3 (niacin), B5 (pantothenic acid); B6 (pyridoxine); B7 (biotin), also known as vitamin H; B9 (folic acid) and B12 (cobalamin). Other substances, such as B10, B11 and B4, are not really vitamins. In addition to their nutritional contents, they have effects on other pathologies, such as pain treatment [2].

2. Chemical Structure

Thiamine or B1 has a thiazole ring and a pyrimidine group, creating together a structure containing sulfur within two rings bonded by a methylene group. It is water-soluble and has an organic cation that, at neutral pH, allows thiamine to go through cellular membranes at normal physiological concentrations, through a specific mechanism called high affinity thiamine transportation. [3] The Figure 1, show the chemical structure of the thiamine.

Pyridoxine or vitamin B6 is water-soluble and stable in both heat and acidic media. The chemical structure of vitamin B6 main compounds (pyridoxine, pyridoxal and pyridoxamine) share a pyridine, hydroxyl and aminophenyl ring, with hydroxymethyl substituents on position 4. In pyridoxine, an alcohol group substitutes the aldehyde group in position 4 of pyridoxal. In pyridoxamine, an amine group replaces the same aldehyde group, see Figure 2. Summarizing, position 5 substitutions are: pyridoxine: -CH_2OH, pyridoxal: -CHO and pyridoxamine: -CH_2NH_2. PLP (pyridoxal 5´-phosphate) acts as coenzyme in all transamination reactions and, in some of them, in decarboxylation and deamination of amino acids [4,5].

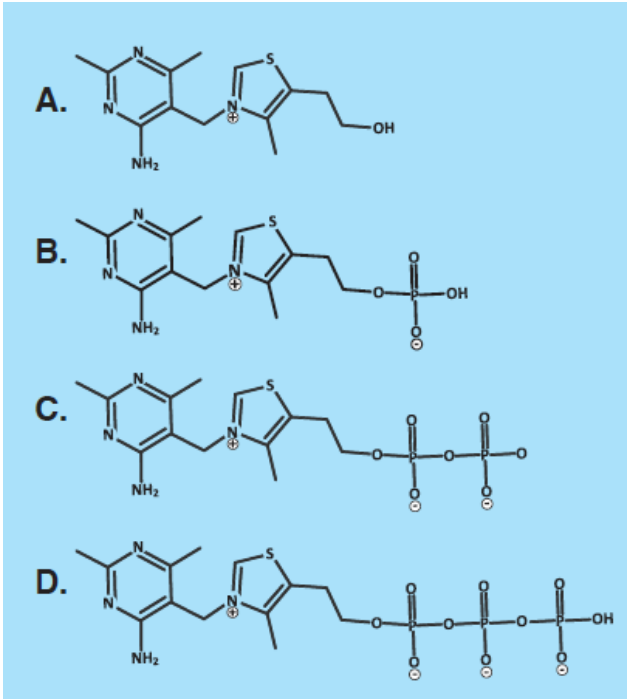

Figure 1. Chemical structure of thiamine

A. Thiamine´s chemical structure, B. Thiamine monophosphate (TMP) C. Phyrophospate (TTP) D. Thriphosphate

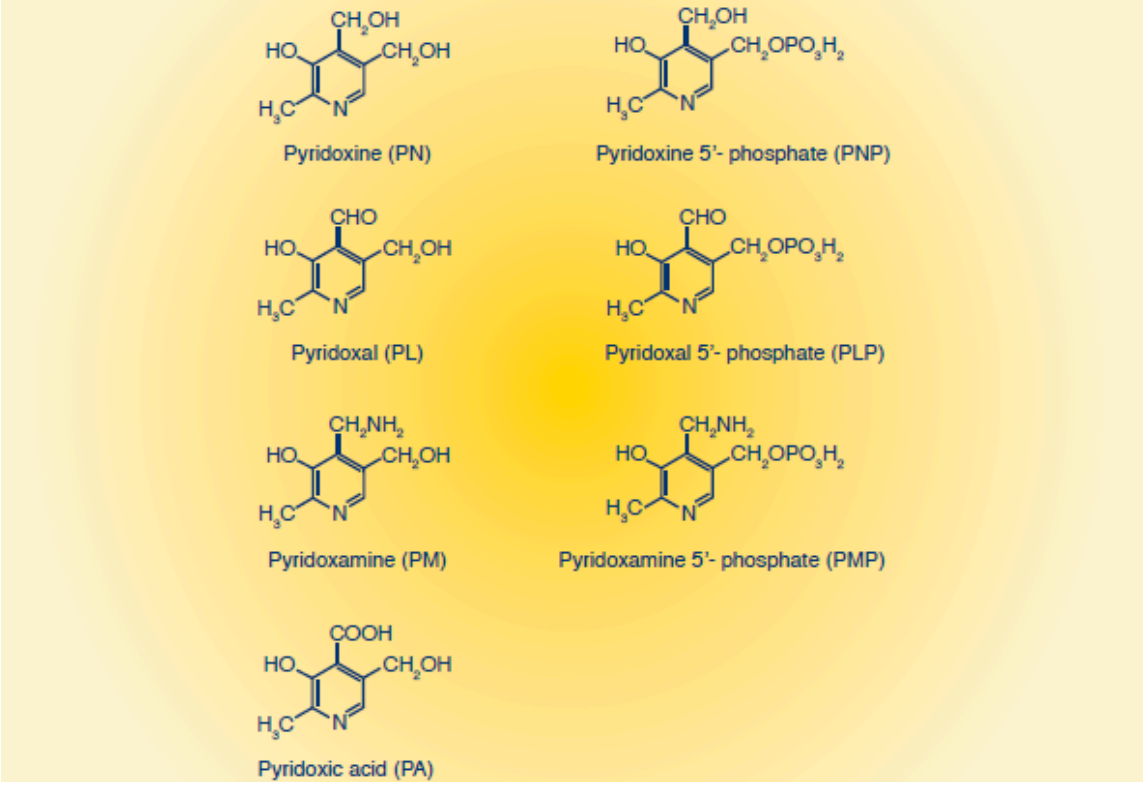

Figure 2. Chemical structure of pyridoxine

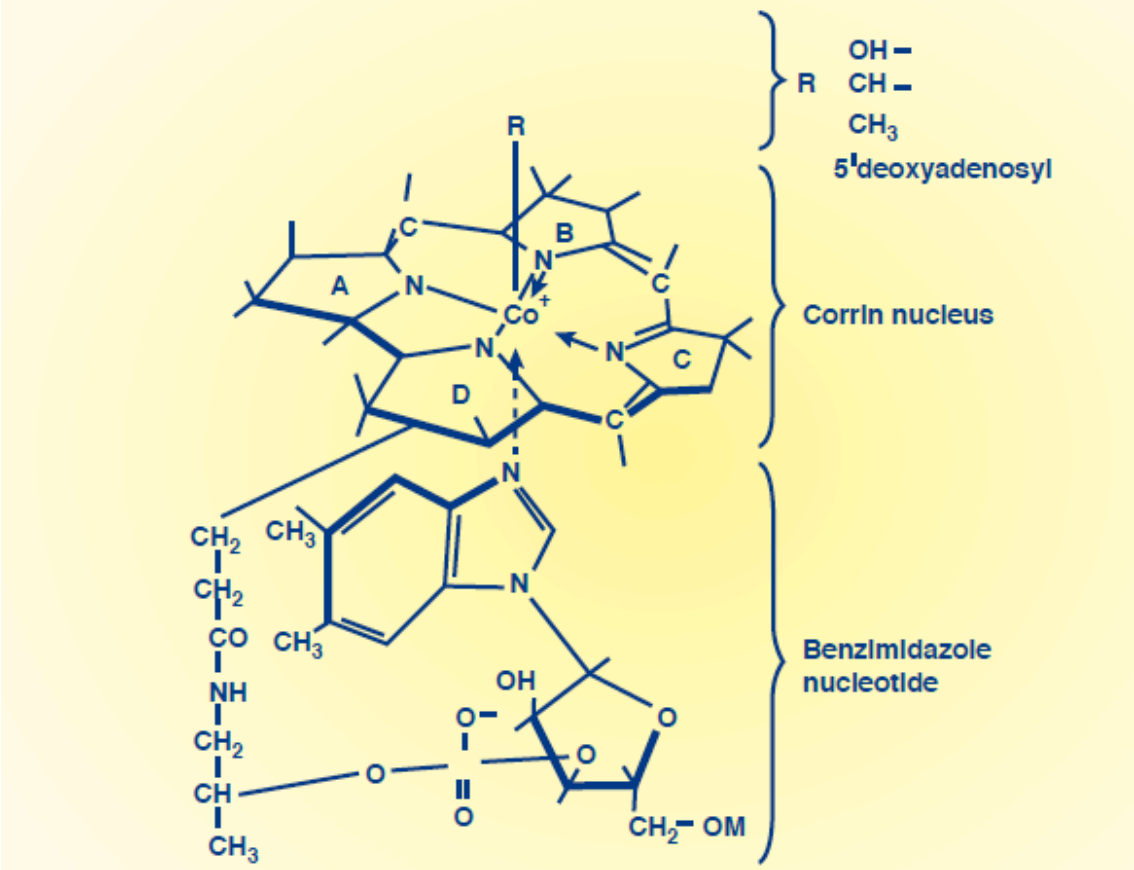

Figure 3. Chemical structure of cobalamin

Cobalamin or vitamin B12 is a cobalamin that belong to the family of complex molecules, formed by the asymmetrical bond of two pyrrolic rings, creating an almost-planar macrocyclic group (corrin nucleus), surrounding a central cobalt atom (Co), see Figure 3 [6].

Cobalamin is an essential co-factor for 2 enzymes: methionine synthase and methylmalonyl coenzyme A mutase (MMCoA). High cobalamin doses can help to maintain low homocysteine and methylmalonic acid (MMA) levels.

3. Thiamine Deficiency: Diagnosis and Clinical Manifestations

Thiamine is necessary for carbohydrate use as an energy source, as well as for amino acid metabolism [7].

Human beings are not capable of synthesizing thiamine and can only store it in small quantities. It is synthesized in bacteria, fungi and plants; it is essential for all mammal diets. Thiamine is found in whole, enriched and fortified products such as bread, cereal, rice, pasta and flour, beef liver and pork meat, milk powder, eggs, legumes, nuts and seed. Dairy products, fruits and vegetables do not contain much thiamine, but, if ingested in large quantities, they can become an important source of this vitamin [8].

Thiamine requirements are related to energy metabolism; it is estimated that a human requires thiamine 0.33 mg per 1000 Kcal of energy. For adults, the recommended daily thiamine dosage is between 1.1 and 1.4 mg per day. Usually, thiamine deficiency develops within 12 months after deficient ingestion [9,10].

Deficient ingestion results from situation restricting its ingestion, requirements increasing (fever, pregnancy), excessive renal loss, anti-thiamine factor consumption (coffee, raw shellfish) or absorption alterations. [11] However, the most common cause of thiamine deficiency worldwide is alcoholism. Causes for deficiency can be predisposing factors (washed rice/malnutrition, GI surgery; gastrostomy, colectomy, gastrojejunostomy, others), psychiatric disorders: nervous anorexia, bulimia, chronic use of alcohol), medical disorders: gastric cancer, colon cancer, severe obesity, chronic diarrhea, pancreatitis, hyperemesis gravidarum, among other cancers, such as lymphoma, leukemia, others), and systemic diseases: renal diseases, acquired immunodeficiency, chronic infectious febrile illness, thyrotoxicosis) [10].

Thiamine deficiency and diabetes mellitus have been shown to be related, since up to 76% of type I diabetics and 75% of type 2 diabetics have low plasma thiamine levels. The polyol pathway increases through hyperglycemia, resulting in the final products of protein glycosylation (AGE), kinase-C protein activation (PKC) and increased flow through the hexosamine pathway [12].

Other cause for deficiency is age. A UK-based study showed that thiamine deficiency prevalence in elders was 8 to 31%, and 23 to 40% in elders from nursing homes. Thiamine biochemical deficiency is 48% in geriatric units [13,14].

B1 deficiency main symptom is heart failure with peripheral edema (wet beriberi) [15], peripheral neuropathy (dry beriberi), and acute (Korsakoff) and chronic (Wernicke) encephalopathy. Heart failure, peripheral neuropathy and cognitive impairment are more frequent in elders [13].

Laboratory diagnosis is complicated, but it can be determined through thiamine blood levels or functional measurement of erythrocyte transketolase activity, [11] as well as measuring metabolites due to alterations on the metabolism of carbohydrates, such as pyruvate or lactate. [10] Thiamine excretion in urine is other option. Thiamine diphosphate-measuring chromatography seems a simple and accurate method to evaluate thiamine status [16].

Nervous system: the best example for thiamine deficiency is Wernicke' syndrome, since this pathology presents with lesions in the cerebral stem, hypothalamus, thalamus and mammillary bodies with glial proliferation, capillary dilation and perivascular hemorrhage, in addition to neurodegeneration, consequence of increased production of nitric oxide synthase, intracellular adhesion molecules-1 (ICAM-1) and oxygen reactive species. [17] Thiamine participates in the metabolism of carbohydrates, both in euglycemic and hyperglycemic environments, with the latter being capable of increasing thiamine consumption. [18] An interesting aspect related to thiamine deficiency in diabetics is that supplements (>4 mg per day) have shown to normalize thiamine levels in red cells, while increased dietary thiamine consumption at the recommended dose (1-1.4 mg/day) does not increase thiamine levels, suggesting the need for higher levels than recommended in diabetic patients, compared to normal individuals [19].

The brain uses glucose as substrate to produce energy. [20] Glucose enters the brain by means of facilitated diffusion through the blood-brain barrier (BHE), when B1 blood-levels are high and through active transport when low. [10] Approximately 30% of glucose absorbed by the brain suffers oxidation, through the tricarboxylic acid cycle. Three enzymatic systems, essential for glucose metabolism in the brain, depend on B1: pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and cytosolic transketolase. These three enzymes are used as cofactors for thiamine pyrophosphate (TPP), which represents 80% of total thiamine in nervous tissues 21. Other important aspect is the fact that thiamine deficiency alters the blood-brain barrier (BHE), which lead to rupture of the latter, generating endothelial impairment and decreased brain energy; all of this from decreased alpha-ketoglutarate dehydrogenase activity [11].

Cholinergic neurons synthesize acetylcholine (Ach) and are especially sensitive to cellular death due to thiamine deficiency. [17] Studies conducted in Wernicke encephalopathy show that neurodegeneration is related to increase production of endothelial nitric oxide synthase (SONe), intracellular adhesion molecules-1 (ICAM-1) and oxygen reactive species [22].

There is a possible sub-diagnosis of B1 deficiency, which can be a factor contributing to cardiac or neurological function degeneration. There is also evidence of thiamine deficiency contributing to non-specific symptoms, very common in elderly patients, such as weakness, mobility impairment and anorexia. Diagnosis can be established by measuring erythrocyte transketolase.

4. Pyridoxine Deficiency: Diagnosis and Clinical Manifestations

Vitamin B6 has an important role in the human nervous system. It can be found in animal tissue as pyridoxal 5-phosphate (most common form) and pyridoxamine 5-phosphate; [5] in vegetables it can be found as pyridoxine and is less bioavailable than in animal tissues. Main sources of vitamin B6 are legumes, nuts, whole bread and beef, although it can be found in all food groups.

All living beings depend on vitamin B6 to exist, however, only microorganisms and plants are capable of synthesizing it *de novo*. All other organisms acquire vitamin B6 through nutrients and interconvert it to its

different forms, and adjust it to their needs. The daily dose recommended is 2 mg/d for men and 1.5 mg/d for women.

Total body reserves of B6 are small, approximately 167mg, [23] of which, 80% is in muscular tissue [24].

Although nowadays evident B6 clinical deficiency is rare, at least in the general population. However, there can be a subclinical deficiency (non-diagnosed) in some individuals, especially in the elderly. [24] Hermann and Cols found B6 deficiency in 23% of subjects between 65 and 75 years old, and 40% in of more than 85. [25] In the SENECA study, 23.3% of elder European population presented deficiency in B6 levels (pyridoxal 5-phosphate, PLP < 20 mmol/L) [26].

Vitamin B6 main function is to act as a coenzyme for amino acid transamination and synthesis. It participates in HEM synthesis and metabolism of homocysteine, glucose, lipids, steroids, production of neurotransmitters and synthesis of DNA/RNA. It can also act as a genetic expression modulator.

The most characteristic symptoms of pyridoxine deficiency are in the nervous system and include irritability, alertness impairment, abnormal movement and seizures. Corticosteroids and thyroid hormones can increase pyridoxine requirements and affect the metabolic process dependant on pyridoxal 5-phosphate. Hydroxitryptamine and dopamine synthesis requires pyridoxal 5-phosphate; therefore, its deficiency has been associated to decreased serotonin synthesis. [27,28] Deficiency of biologically active vitamin B6 (pyridoxal 5-phosphate) has been associated to Alzheimer' and Parkinson' disease. Risk factors are advanced age, malnutrition, hospitalization, chronic renal impairment, renal transplant, catabolic status, hemodialysis, peritoneal dialysis, celiac disease, alcoholism, sideroblastic anemia, pregnancy, physical exercise, among others. [24] It has also been associated to oxidative stress inhibition due to amyloid beta Cu (II) [29].

Diagnosis is established by measuring vitamin B6 direct concentration, in plasma or erythrocytes, although it can also be done by determining pyridoxal 5-phosphate metabolite in urine, 4-pyridoxic acid.

Vitamin B6 clinical deficiency treatment requires a mega dose of this vitamin via oral route, usually 50 mg/day, administered depending on the cause of the deficiency. It is important to take into account the subclinical deficiency status, since it has been associated to chronic illness, especially in the elderly, and therefore supplementation of this group is indicated [24].

5. Cobalamin Deficiency

The definition of vitamin B12 deficiency varies greatly; therefore, values obtained in population studies are used to obtain normal serum values. [6] Prevalence of B12 deficiency is unknown in general population; however, incidence seems to increase with age. [30] Framingham study reported a prevalence of B12 deficiency (defined as a serum level under 200 pg/mL and increased levels of homocysteine, methylmalonic acid or both) in 12% of 548 elders of the community. [31] Another study reported 15% of adults more than 65 years old had evidence of vitamin B12 deficiency. [30] Among risk factors for vitamin B12 deficiency are decreased ileum absorption (Crohn disease, ileum resection), decreased intrinsic factor (atrophic gastritis, pernicious anemia, postgastrectomy syndrome), genetics (transcobalamin II deficiency), inadequate ingestion (alcohol abuse, elderly, vegetarians) and chronic drug use (H2 blockers, metformin, proton pump inhibitors) [32].

Vitamin B12 is found in a wide variety of foods of animal origin. The plant foods do not contain B12, unless they are fortified. Entrails (beef liver), shellfish (clams), beef and bird meat, eggs, milk and other dairy products, some cereals and nutritional yeast contain B12 [33].

B12 minimum plasma level must be over 200 pg/mL, however, clinical manifestations have been observed with levels between 200 and 300 pg/mL. [34] Van Goor et al [35] suggests measuring methylmalonic acid (AMM) and homocysteine (Hcy) in case of high suspicion of cobalamin deficiency.

Total body reserves of cobalamin are 2-5 mg, of which half is stored in the liver. Daily recommended dose is 2 µg/day in adults, 2.6 µg/d in pregnant and breastfeeding women, and 2 µg/d in teenagers. Neurological disorders due to vitamin B12 deficiency typically occur in both sexes between 40 and 90 years old, with a peak between 60-70 years [36].

B12 deficiency is associated with skin (hyperpigmentation, vitiligo), gastrointestinal (glositis, jaundice), hematological (macrocytic and megaloblastic anemia), and neuropsychiatric (cognitive impairment, gait disorder, peripheral neuropathy, depression) manifestations. [32] It is important to mention that the liver stores large quantities of B12, reason why it can be a delay of 5 to 10 years between the deficiency starting and the clinical symptoms appearing [37].

Pernicious anemia is characterized by autoimmune atrophic chronic gastritis and represents a common cause of B12 deficiency.

Neurological deficiencies include paresthesia, peripheral neuropathy, demyelination of the corticospinal tract and dorsal column (subacute combined degeneration). Both glial cells, myelin and interstitium are the main structures affected by B12 deficiency. Specifically, astrocytes and microglia are responsible for increasing cell numbers for glial fibrillary acidic protein, and production and secretion of growth and cytokine factors. Damage to myelin affects particularly the lamellae, which disorganizes due to edema. [38] In the deficiency status there is a decrease in epidermal growth factor production, which is a myelotrophic factor [39].

A key interaction between B12 and folate exists in methionine synthesis from homocysteine, via methionine synthase, in which 5-methylhydrofolate and methyl-vitamin B12 are cofactors in a reaction that can be inhibited by nitric oxide. The folate cycle, whose function is to synthesize methyl groups, is essential for many genomic and non-genomic methylation reactions via the S-adenosylmethionine pathway and indirectly for synthesizing purine and thymidine, and therefore, DNA and RNA nucleotide.

Subacute combined degeneration (DCS): includes paresthesia, ataxia, loss of vibration and proprioception, abnormal reflexes, urinary and bowel incontinence, optic atrophy, orthostatic hypotension and autonomic disorders [6].

Neuropathy: most series evaluating complications of cobalamin deficiency cannot distinguish between myelopathy and neuropathy, since from a clinical point of view; both

cases can have paresthesia, weakness and numbing. However, findings of superior motor neuron syndrome: Babinski and sensitive spinal level indicate myelopathy, and decreased or absent muscular stretch reflex suggest peripheral neuropathy. Appearance of symptoms in the hands, as well as involvement of both upper and lower limbs and sudden appearance of symptoms are manifestations suggesting peripheral neuropathy due to cobalamin deficiency [41].

Dementia: cobalamin deficiency has been associated to neuropsychiatric symptoms and Alzheimer disease. It is estimated that more than 40% of the elderly have B12 deficiency (cobalamin), most due to cobalamin malabsorption.

Complex B effects on pain treatment.

Each one of complex B vitamins (B1, B6, and B12) have individual effects on several pain pathways, although they have shown to be beneficial when combined. The benefits of these vitamins in neuropathic pain treatment, for example, can be divided in two aspects: those decreasing damage effects on the nervous fiber and those that are antinociceptive and antihyperalgesic [18].

Thiamine (B1). *Song SX and cols,* showed in animal models that B1 modulates neural excitability and sodium currents in damaged neurons in the dorsal root ganglion (GRD), suppressing therefore thermal hyperalgesia and contributing to pain control. [42] Likewise, it has effects on neuronal conduction and excitability [43].

Synthesis and secretion of serotonin are also increased in various areas of the brain; in addition to reducing thalamic neuron response to nociceptive stimulation [44].

Intratecal administration of B1 induces hyperalgesia inhibition, which can be reverted by blockers via cyclic guanosine monophosphate (GMPc)-protein kinase G (PKG) signaling. [45] This route has antinociceptive activity in pain processing [46].

An important effect is through activation of the nitric oxide (ON)-cyclic GMP (GMPc) pathway. ON activates soluble guanylate cyclase (Gcs), which generates GMPc, activating in turn type G protein kinase (PKG), hyperpolarizing nociceptor potassium channels, [44] See Figure 4.

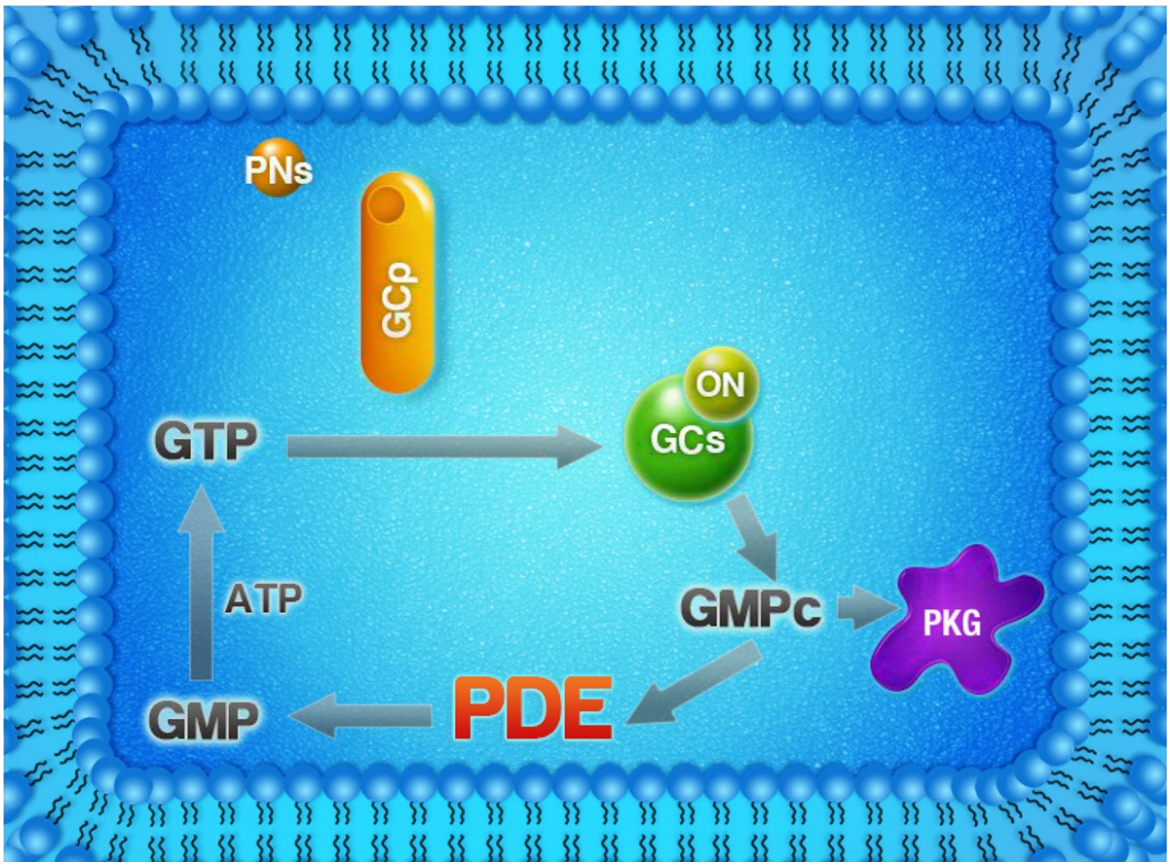

Figure 4. Action of thiamine on the ON-cyclic GMP pathway for pain treatment

GMP: guanosine monophosphate, PDE: phosphodiesterase, GMPc: cyclic GMP, PKG: Protein Kinase G, ATP: Adenosine triphosphate, GCs: Soluble guanylyl cyclase, GTP: Guanosine triphosphate, ON: Nitirc oxide.

Pyridoxine (B6). It can interact with intra - supraspinal receptors, with both endogenous opioids tonically discharged and non-opioid inhibitory neurotransmitters, such as serotoninergic systems and gamma aminobutyric acid (GABA). [47,49] Pyridoxal 5-phosphate participates in dopa to dopamine conversion and allows conversion of glutamate to the inhibitory GABA neurotransmitter, preventing neurotoxicity-related damage [5,50].

It can alter intracellular glutamate levels and cell surface calcium channels, [47,51] inducing hyperalgesia inhibition.

Cobalamin (B12).

Cobalamin effects are based in remyelination. The mechanism of damage to the nerve is related to B12 deficiency itself, as well as with production of metabolites related to the latter (increased methylmalonic acid) and decreased S-adenosylmethionine (SAM), which participates in Hcy conversion to useful metabolites. In addition, B12 is necessary as methyl donor in polyamine synthesis and transmethylation reactions. [52] Figure 5 shows mechanisms related to the damage process to the nervous fiber.

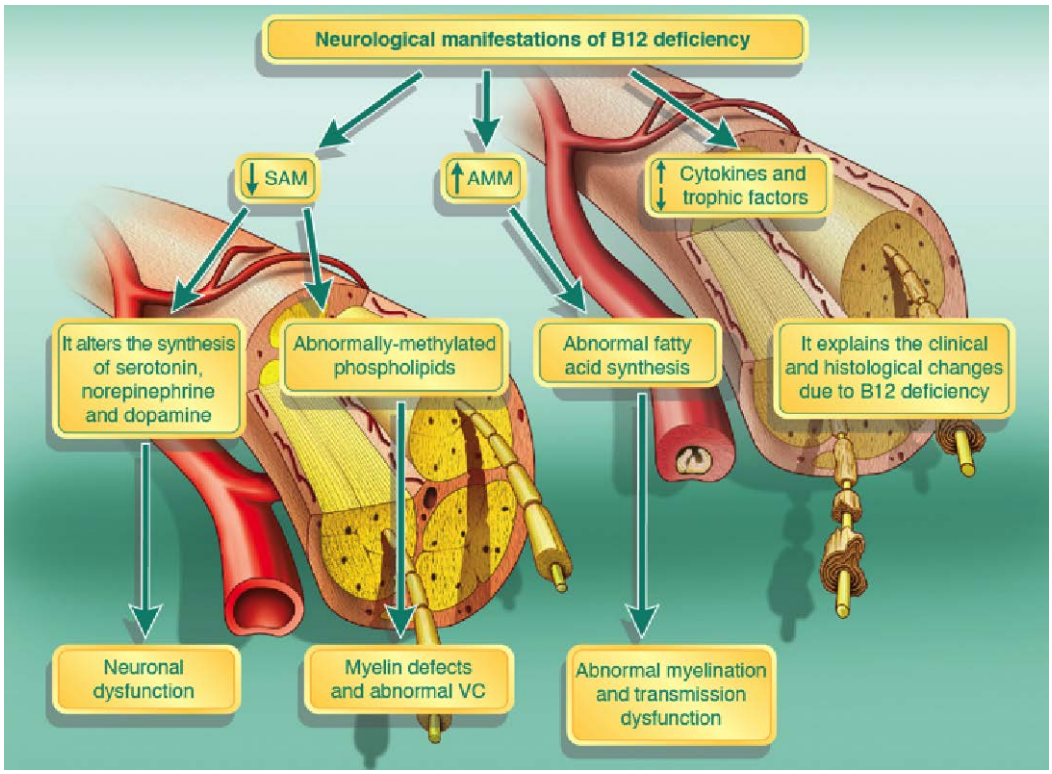

Figure 5. Effects on the nervous system, related to B12 deficiency

SAM: S-adenosylmethionine, AMM: methylmalonic acid.

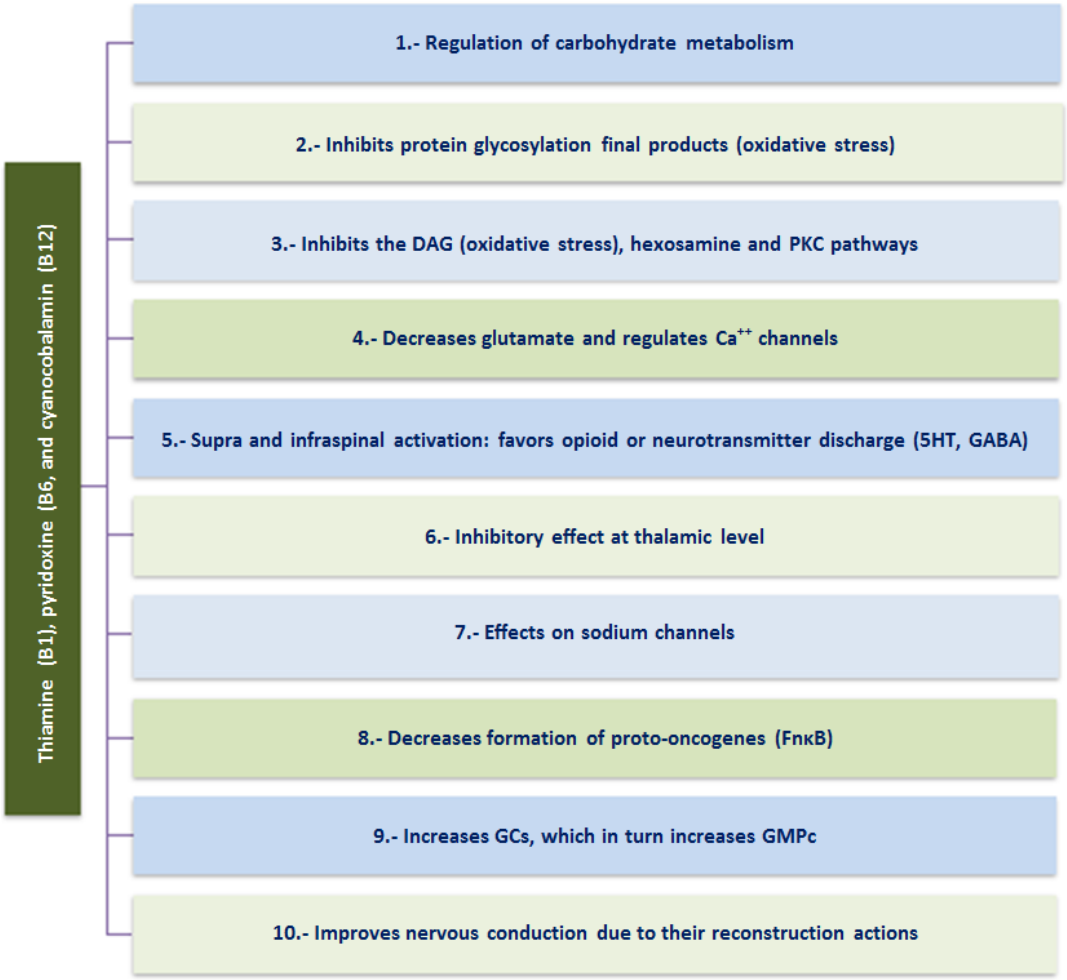

Figure 6. Physiologic effects summary of complex B vitamins on pain treatment

DAG: diacylglycerol, PKC: protein kinase, 5HT: serotonine, GABA: gamma aminobutyric acid, FNκB: nuclear factor kappa-Beta GCs: soluble guanylate cyclase, GMPc: cyclic GMP.

Likewise, cobalamin deficiency is associated with increased nuclear factor kappa-Beta (FN-kB), which increased inflammation, since this factor increases tumor necrosis factor alpha (FNT- α) levels, immune reactions and apoptosis [38].

Mechanisms to decrease nervous fiber damage are based on decreasing the final products of advanced protein glycosylation, [53] inhibition of the diacylglycerol (DAG) pathway, which is a potent protein kinase (PKC) activation pathway. [54] Likewise, it also regulated the hexosamine pathway, regulating and improving the pentose pathway through alternate metabolism pathways [18].

Global effects of combining thiamine and cobalamin.

The analgesic action of vitamins B1, B6 and B12 is based on:

* Improving axonal conduction, which might contribute to analgesia. [43]

* Activation of soluble guanylate cyclase (Gcs) through ON and therefore increasing GMPc synthesis in various tissues. [44]

* Inhibition of thermal hyperalgesia (not mechanical). [47]

* Reconstructive activation in damaged nerves, contributing to nerve function recovery. [55]

* Decreased intracellular glutamate levels and cellular surface of calcium channels. [51]

* Regulation of FN-kB. [38]

* Decreased nervous fiber damage due to a metabolic disorder: decreased oxidative stress, decreased activation of both the DAG-PKG pathway and hexosamine pathway. [12]

* Regulation of carbohydrate metabolism. [18]

Figure 6 shows the global effects of complex B vitamins and Figure 4 shows individual effects of each vitamin on several physiopathological pain mechanisms.

6. Conclusions

Complex B vitamins have a vital role in human beings, since they participate in the metabolism of carbohydrates and amino acids, acting as enzymatic cofactors in multiple synthesis reactions.

Although traditionally it has been considered that only severe deficiencies cause symptoms, many common diseases, such as diabetes mellitus, surgical procedures, among others, can produce subclinical deficiency of vitamin B1, B6 or B12, so it is important to consider these deficiencies as possible factors that could contribute to or exacerbate certain complications of these diseases. Also, aging is a factor that may alter the absorption of these vitamins, through use of multiple drugs or decreased specific factors necessary for the absorption of these. Therefore, they should be considered clinical and subclinical deficiencies as possible manifestations neurological disorders, such as painful neuropathy.

Today, it is possible to explain the analgesic effect of B vitamins through their effects on activation of the nitric oxide (ON)-cyclic GMP (GMPc) pathway, decreasing the levels of intracellular glutamate and NF-kB, improving axonal conduction (which contributes to analgesia), restoring the nerve fiber and inhibiting thermal hyperalgesia.

Therefore, it is necessary to know the effects that these vitamins because they have further shown to be an adjunct treatment option for neuropathic pain, in combination with gabapentin or in nociceptive pain in combination with diclofenac. Undoubtedly, more clinical studies to demonstrate the effect on pain management are needed to extend its recommendation in this pathology

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