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The vitamin B6 paradox: Supplementation with high concentrations of pyridoxine leads to decreased vitamin B6 function

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ABSTRACT

Vitamin B6 is a water-soluble vitamin that functions as a coenzyme in many reactions involved in amino acid, carbohydrates and lipid metabolism. Since 2014, > 50 cases of sensory neuronal pain due to vitamin B6 supplementation were reported. Up to now, the mechanism of this toxicity is enigmatic and the contribution of the various B6 vitamers to this toxicity is largely unknown.

In the present study, the neurotoxicity of the different forms of vitamin B6 is tested on SHSY5Y and CaCo-2 cells. Cells were exposed to pyridoxine, pyridoxamine, pyridoxal, pyridoxal-5-phosphate or pyridoxamine-5-phosphate for 24 h, after which cell viability was measured using the MTT assay. The expression of Bax and caspase-8 was tested after the 24 h exposure. The effect of the vitamers on two pyridoxal-5-phosphate dependent enzymes was also tested.

Pyridoxine induced cell death in a concentration-dependent way in SHSY5Y cells. The other vitamers did not affect cell viability. Pyridoxine significantly increased the expression of Bax and caspase-8. Moreover, both pyridoxal-5-phosphate dependent enzymes were inhibited by pyridoxine.

In conclusion, the present study indicates that the neuropathy observed after taking a relatively high dose of vitamin B6 supplements is due to pyridoxine. The inactive form pyridoxine competitively inhibits the active pyridoxal-5′-phosphate. Consequently, symptoms of vitamin B6 supplementation are similar to those of vitamin B6 deficiency.

1. Introduction

Vitamin B6 is a water-soluble vitamin found in e.g. meat, poultry, fish, legumes, bananas, nuts and cereals (Ueland et al., 2015). Vitamin B6 functions as a coenzyme in many reactions that are involved in amino acid, carbohydrates and lipid metabolism (Eliot and Kirsch, 2004). Additionally, vitamin B6 also plays a role in neuronal signaling through the synthesis of neurotransmitters (Percudani and Peracchi, 2009). The major forms of vitamin B6 are: pyridoxine, pyridoxal, pyridoxamine and their phosphorylated derivatives pyridoxine 5′-phosphate, pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate (Ueland et al., 2015).

Vitamin B6 is mainly present in our diet as pyridoxine, pyridoxal and pyridoxamine. After passive absorption in the intestine, the major part of the vitamin is converted in the liver to pyridoxal-phosphate (Merrill et al., 1978; Zuo et al., 2015). After hydrolysis to pyridoxal by alkaline phosphatases, pyridoxal-phosphate becomes available for every cell in our body. Within the cells, pyridoxal is phosphorylated by pyridoxal kinase to pyridoxal-phosphate (Albersen et al., 2015). Most of the pyridoxal in excess of tissue requirements is oxidized by the liver to 4-pyridoxic acid (4-PA), which is the major degradation product of vitamin B6 in the urine. The metabolic routes of vitamin B6 are shown in Fig. 1.

Vitamin B6 deficiency may arise from a too low intake, malabsorption, or due to drugs that inhibit enzymes involved in pyridoxal-phosphate metabolism. Low levels of vitamin B6 are found in elderly, individuals with traumatic femoral fractures, and in alcoholics (di Salvo et al., 2012). Vitamin B6 deficiency has been related to increased risk of cardiovascular diseases and polyneuropathy, which is the general term for damage to peripheral nerves, causing symptoms as weakness, numbness and pain in the extremeties (Spinneker et al., 2007). In these cases, vitamin B6 supplements are advised (Kashanian et al., 2007; Wyatt et al., 1999; Bendich, 2000). In the US, about 28%–36% of the general population uses supplements containing vitamin B6 (Bailey

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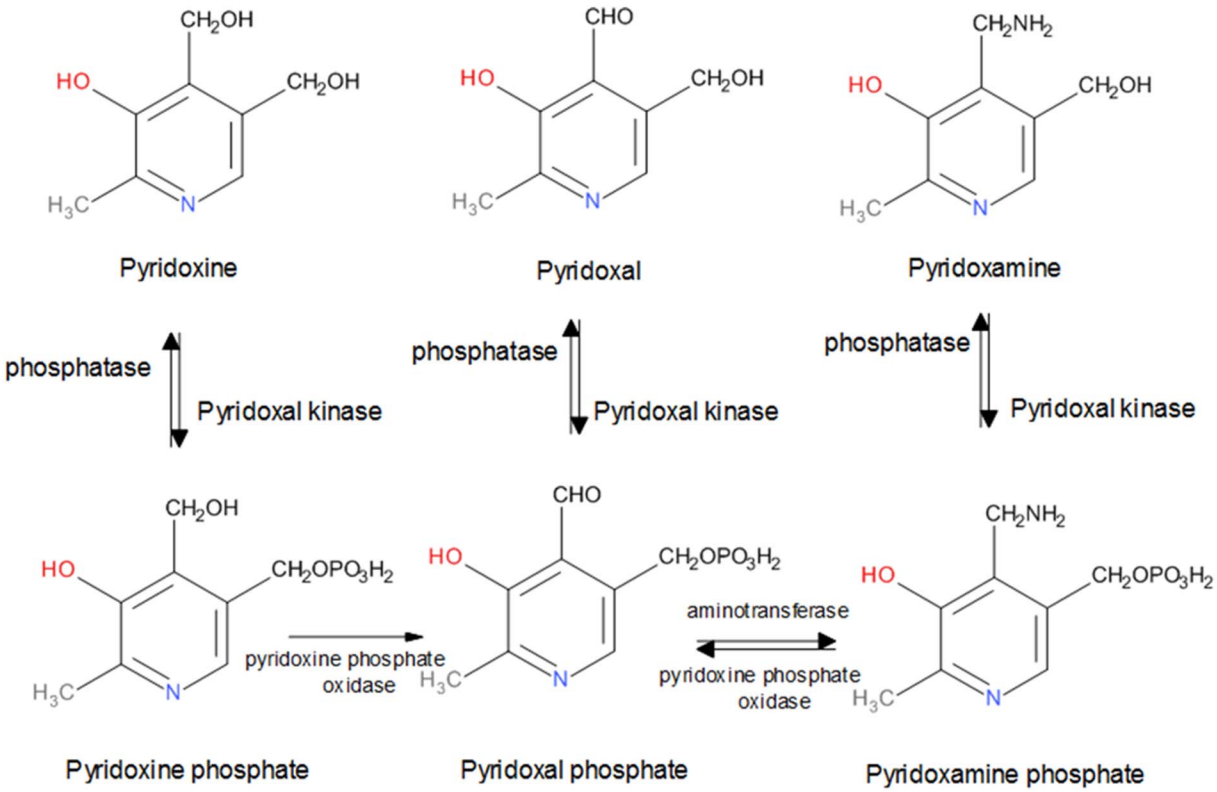

Fig. 1. Vitamin B6 metabolism.

et al., 2011; Morris et al., 2008). These supplements are most often taken by children up to 6 years and by adults over 51 years of age. Most vitamin B6 supplements contain pyridoxine ranging from 25 to 100 mg/tablet.

Recently, the European Food Safety Authority (EFSA) has established an upper limit (UL) of 25 mg/day. This UL was based on neurological complaints observed after taking 50 mg of pyridoxine per day. This is 4 times lower than the previous UL of 100 mg/day of USDA and other authorities. However, since vitamin B6 is considered to be relatively safe, doses of over 2000-fold the recommended dietary allowance of 1.4–2.1 mg/day (depending on sex and age) are used in some circumstances (Simpson et al., 2011).

Paradoxically, supplementation of vitamin B6 has also been shown to lead to polyneuropathy. Already in 1987, a case of polyneuropathy after supplementation of a high dose of vitamin B6 was reported (Schaumburg et al., 1983). In the years that followed, more cases on vitamin B6 toxicity were reported. Recently, The Netherlands Pharmacovigilance Centre Lareb, which collects and analyses reports of adverse reactions of medicines and vaccines, published a report concerning the side effects of vitamin B6 supplements. Since 2014, > 50 cases of sensory neuronal pain due to vitamin B6 were reported to Lareb (Lareb, 2015). The dose of vitamin B6 differed from 1.5–100 mg. In these cases, high plasma levels of pyridoxal-phosphate between 183 nM and 4338 nM were found. Plasma levels of pyridoxine were not determined. Up to now, the mechanism of this toxicity is enigmatic and the contribution of the various B6 vitamers to this toxicity is largely unknown. Our hypothesis is that pyridoxine is the vitamer mainly responsible for the vitamin B6 induced toxicity. Pyridoxine, which is not the active form, is ultimately converted into pyridoxal-phosphate, which is the active form. High levels of pyridoxine are thought to inhibit pyridoxal-phosphate -dependent enzymes by competing with the bioactive vitamer, pyridoxal-phosphate.

In the present study, the neurotoxicity of the different forms of vitamin B6 is tested on neuroblastoma cells (SHSY5Y). A second cell line (CaCo-2) was used to determine if the effects of vitamin B6 are specific for neuronal cells. In line with our hypothesis, pyridoxine showed the highest toxicity. The paradox that the vitamin B6 pyridoxine vitamer in high concentrations inhibits vitamin B6 dependent processes was confirmed using vitamin B6 dependent enzymes tyrosine decarboxylase and alanine transaminase.

2. Materials and Methods

2.1. Chemicals

L-alanine, α-ketoglutarate, alanine transaminase, lactate dehydrogenase (LDH),NADH, pyridoxine, pyridoxal, pyridoxamine dihydrochloride, pyridoxal-phosphate hydrate, pyridoxamine -phosphate, Toluene, tyrosine decarboxylase apoenzyme (1.1 U/mg) from Streptococcus faecalis, L-tyrosine and picrylsulfonic acid were all obtained from Sigma (St. Louis, USA).

2.2. Tyrosine decarboxylase enzyme activity

Tyrosine decarboxylase activity was measured according to the tyrosine decarboxylase enzyme activity assay. In this assay, tyrosine is converted into tyramine by the enzyme tyrosine decarboxylase and the co-enzyme pyridoxal-phosphate. At first, tyrosine (0–100 μM) was added to a tube containing tyrosine decarboxylase (1.1 U/mL) with pyridoxal-phosphate and acetate buffer (pH 5.5). This mixture was incubated for 10 min at 37 °C. Then, 1 mL of K_2CO_3 (1 M) was added to the tube, which was followed by the addition of 1 mL of TNBS (10 mM). Finally, 2 mL of toluene was added, after which the tube was vortexed for 20 s. After centrifugation for 5 min at 2000 RPM, the enzyme activity was determined by measuring the absorbance at 340 nm using a spectrophotometer. In order to determine the effect of the different forms of vitamin B6, the enzyme was first incubated together with the vitamer of vitamin B6 (at 5 μM) for 2 min. Then, the same protocol was followed as described above.