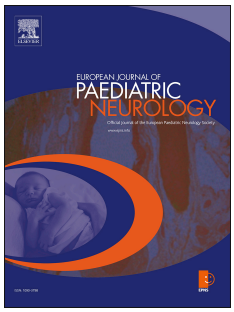

A vitamin a day keeps the doctor away: The need for high quality pyridoxal-5′-phosphate

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A Vitamin a Day Keeps the Doctor Away: The Need for High Quality Pyridoxal-5'-Phosphate

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Abstract

Background - A rare subset of vitamin B6 responsive seizure disorders does not respond to pyridoxine, and requires the active form of vitamin B6, pyridoxal-5'-phosphate (PLP), to maintain seizure control. Patients with PLP-responsive seizures are dependent on chronic PLP treatment, yet no licensed PLP product is available. PLP food supplements, a product category regulated less stringently than medication, may prove of insufficient effectiveness and safety. Here we describe and discuss three patient scenarios which illustrate this conundrum.

Methods - Medical and laboratory records were reviewed with retrospective extraction for three unrelated patients who suffered complications during treatment with PLP food supplements.

Results – Two cases of PNPO deficiency and one case of PLP-dependent epileptic encephalopathy without a (genetic) diagnosis are reported. These patients are critically dependent on PLP for seizure control and have suffered complications due to insufficient quality of these food supplements during the course of treatment. Complications include the occurrence of seizures following the administration of suspected low quality PLP, inactive PLP due to light exposure, a PLP intoxication, resisting administration and post-administration vomiting as a result of the ingestion of large amounts of capsules per day.

Conclusion – This case series illustrates that the reliance on food supplements as anti-seizure therapy is not without risk. The treatment of PLP-dependent seizures exemplifies that PLP is administered as medication, thus there is a clear need for licensed vitamin products of pharmaceutical quality.

Key words: pyridoxal-5'-phosphate, PNPO deficiency, vitamin B6 disorders, seizure disorders, neonatal epileptic encephalopathy

1. Introduction

For many inherited metabolic disorders (IMDs)*, caused by monogenic defects in biochemical pathways and processes, vitamins acting as co-factor or chaperones are key components of effective therapy. Literally, the proverb 'a vitamin a day keeps the doctor away' applies, as insufficient administration can result in relapse of seizures and encephalopathy or cause other deterioration. A well-known group of such IMDs are vitamin B6-responsive seizure disorders, classically presenting during the neonatal or infantile period with intractable epilepsy. Indeed, for PDE-ALDH7A1 (MIM: 266100), TNSALP deficiency (MIM: 171760), hyperprolinemia type 2 (MIM: 239510) and more recently in *GOT2* (MIM: 138150), encoding the PLP-dependent enzyme mitochondrial aspartate aminotransferase, and *PLPBP* deficiency (previously termed PROSC, MIM: 604436), pyridoxine therapy is sufficient to control seizures. This form of vitamin B6 is safe, available, and affordable both in oral and iv form.[1] However, a subset of vitamin B6 responsive seizure disorders is intractable to pyridoxine, requiring the active form of vitamin B6, pyridoxal-5'-phosphate (PLP). A PLP-responsive polyneuropathy precipitated by *PDXK* mutations was also recently identified.[2]

In 2002, Kuo et al. first described PLP-responsive epilepsy in an infant with seizures resistant to pyridoxine.[3] Shortly thereafter, pyridoxamine-5′-phosphate oxidase (PNPO) deficiency (MIM: 6032870) was established as the underlying cause for this form of epilepsy.[4] More recently, *PLPBP* mutations have also been shown to precipitate seizures that respond to either pyridoxine or PLP treatment.[5] PLP is the biologically active form of vitamin B6. It is highly reactive and a cofactor for more than 140 enzyme-catalyzed reactions, mainly in the central nervous system.[6,7] PLP is mainly obtained by recycling dietary vitamin B6 through a so-called salvage pathway, including oxidation of phosphorylated vitamin B6 by the enzyme

* Abbreviations: AADC: aromatic l-amino acid decarboxylase; AASA: alpha-aminoadipic semialdehyde; IMDs: inherited metabolic disorders; PLP: pyridoxal-5'-phosphate; PLPBP: pyridoxal phosphate binding protein; PNPO: pyridoxamine-5′-phosphate oxidase; PDE-ALDH7A1: pyridoxine-dependent epilepsy due to *ALDH7A1* mutations; TNSALP: tissue-nonspecific alkaline phosphatase.

PNPO.[8] In PNPO-deficient patients, this PLP salvage pathway is therefore impaired. The biochemical mechanism of disease in *PLPBP* mutations is less well understood but is thought to relate to an impaired PLP homeostasis. [5,9,10]

Patients with the classical phenotype of PNPO deficiency present with intractable neonatal seizures or infantile spasms, which sometimes respond to pyridoxine but usually require PLP treatment.[6,11] Most genetically confirmed PNPO-deficient patients suffer from intellectual and/or motor disabilities– sometimes prevented by early administration PLP.[11,12] Other clinical features reported include premature birth and hepatotoxicity, the latter being debated as a side effect of PLP treatment.[6,11] In high doses PLP may be toxic, and this is true for degraded PLP as well, which can be the result of light exposure.[6,13,14]

Patients with *PLPBP* mutations have a variable clinical picture including neonatal or early childhood epileptic encephalopathy, a lethal mitochondrial encephalopathy and a movement disorder mimicking aromatic l-amino acid decarboxylase (AADC) deficiency (MIM: 608643) have been described.[5,9] This variation is not surprising given that PLP as a cofactor is required for essential pathways of mitochondrial metabolism and AADC itself.[15-17] Several other components that may be relevant to our understanding of vitamin B6-related disorders – including the mechanism of maintaining PLP homeostasis in human cells – are not yet fully understood.[17]

Regardless of the underlying defect, patients with PLP-responsive seizures are dependent on chronic PLP treatment to avoid status epilepticus, regression, coma and even death. So far, no licensed PLP product is available and patients depend on PLP in the form of food supplements.[14] In contrast to medicinal products, which are tightly regulated, these supplements are subject to less stringent food safety regulations. Recently, major concerns rose regarding the dose accuracy, stability and safety of these PLP supplements.[14] Here we present three unrelated patients with PLP-dependent epilepsy who suffered complications during the

course of treatment with PLP of food supplement quality. These alarming scenarios emphasize the need for licensed products of pharmaceutical quality – not only for PLP, but also for other vitamins which are the mainstay of therapy for at least 35 vitamin-responsive IMDs.[18]

2. Materials and methods

Parents of the patients provided written informed consent for publication of this case series. Medical and laboratory records were reviewed with retrospective data extraction (2015-2021).

3. Results

An overview of the patient history, clinical features and PLP treatment characteristics for the three patients described below are provided in Table 1. Details of the PLP food supplements prescribed are listed in Table 2.

3.1 Case 1.

This 4-year old male patient with a genetically confirmed diagnosis of PNPO deficiency has been dependent on PLP monotherapy (34 mg/kg/d) to control his seizures. Prior to diagnosis and initiation of PLP treatment, this boy was born in the Netherlands as the first and only child to nonconsanguineous Chinese parents. He presented with seizures within the first hours of his life. Treatment with therapeutic doses of anticonvulsive therapy was started immediately and included phenobarbital, midazolam, levetiracetam and lidocaine, but did not result in adequate seizure control. The addition of pyridoxine (first 50 mg/kg/d, later 22 mg/kg/d, iv) also had no effect. At day three, a PLP trial (30 mg/kg/d, through nasogastric tube) was initiated resulting in resolution of seizures. Biochemical analysis was consistent with PNPO deficiency (CSF: pyridoxal <20 nmol/l, ref. 20-93, PLP not detectable, ref. 30-49 nmol/l; urine alpha-aminoadipic semialdehyde (AASA) 469 nmol/mmol creatinine, ref. 270-2850). The boy was

discharged on an increased dose of PLP monotherapy (40 mg/kg/d). Molecular testing confirmed the diagnosis of PNPO deficiency, revealing compound heterozygous *PNPO* mutations (paternal c.364-1G>A; p.(?), maternal c.448_451del; p.ProArgfs*27), which have been reported previously.[4,19] The c.364-1G>A variant is considered pathogenic due to its location in the splice acceptor site of exon 3, resulting in a splicing defect. Its effect at the protein level has not been analyzed.

Following this diagnosis, he was treated relatively successfully with a food grade PLP supplement (50 mg tablets by Bonusan) during the first year of life.[20] Potential photochemical degradation was a concern, as his parents crushed and dissolved the tablets before administration though a nasogastric tube.[14] Despite this, he remained seizure free except for one seizure during febrile illness. In 2018, a Dutch legal limit on vitamin B6 supplements was introduced, maximizing PLP dosage in supplements at 21 mg per day.[21]

Several brand switches followed, with serious consequences in two instances: after switching to 20 mg capsules (by AOV, labelled as containing 20 mg vitamin B6 but 35,1 mg PLP) the parents observed abnormal behavior, followed by seizures.[22] Although another supplement (20 mg PLP+ tablets by Bonusan) was effective, this contained additional substances (vitamin B12 and folic acid), which rendered them unsuitable for long term use.[20] Another brand (20 mg capsules by Vitals) required daily intake of 30-40 capsules, pushing for a search to another supplement (100 mg capsules by Life Extension®) at higher concentration which, however, again resulted in abnormal behavioral characteristics. Patient immediately became more fatigued and less alert; several weeks later, he showed the behavioral changes parents recognized as the prodromal symptoms of a seizure (wandering around the room with no signs of contact between him and the parents).[23,24] Therefore, he remains on the Vitals supplement, but ingestion of the required 33 capsules is a daily struggle. A moderate global developmental delay was observed in this patient. His transaminases are checked routinely, and

elevation at age 2 (ASAT: 83 U/l, ref. 0–60, ALAT 59 U/l, ref: 0-45) prompted reduction of PLP dosage, after which transaminases have nearly normalized (most recent at age 4 ASAT: 40 U/l, ref. 0–40, ALAT 57 U/l, ref: 0-45).

3.2 Case 2.

This 6-year old male child with genetically confirmed PNPO deficiency has been on PLP monotherapy (31 mg/kg/d) for seizure control since the age of 17 months. Up until that point, he had been treated with pyridoxine instead of PLP. The initial clinical details of this case have been reported previously.[25] In summary, he was born at term to healthy first-degree cousins of Pakistani decent. He first presented with seizures one day after birth, which proved refractory to phenobarbital, midazolam and lidocaine. A pyridoxine trial (15 mg/kg/day, iv) showed a delayed response as his seizures disappeared after one day. Subsequently, all antiepileptic medications were successfully withdrawn. Biochemical analysis supported the diagnosis of PNPO deficiency rather than pyridoxine-dependent epilepsy (CSF obtained on pyridoxine treatment: pyridoxal 297 nmol/l, ref. 5 -106 nmol/l, PLP 21 nmol/l, ref 11 -46, pyridoxamine not quantified but elevated; Urine: AASA 0.5 mmol/mol creatinine, ref. 0 -2.0). *PNPO* analysis revealed a previously unreported homozygous missense mutation (c.481C>T; p.Arg161Cys); parents are heterozygous carriers. Despite this, pyridoxine monotherapy was continued at first in favor of switching to PLP. This was decided due to the evident pyridoxine-responsiveness observed in the patient, the risk of a paradoxical reaction following the replacement of pyridoxine by PLP and the potential liver toxicity linked to PLP therapy.[25]

At 17 months old however, pyridoxine was replaced by treatment with PLP (30 mg/kg/day) following an escalation in seizure frequency and two instances of status epilepticus. PLP monotherapy (50 mg capsules by Solgar) induced effective seizure control.[26] At 3.5 years old, a seizure occurred that likely resulted from degradation and subsequent reduced activity of

the PLP due to light exposure, since the protective film surrounding these capsules was routinely cut open to dissolve the PLP before administration.[14] Several brand switches followed, without serious consequences. The first brand (20 mg tablets by AOV) was withdrawn following concerns over their PLP content, the second (20mg PLP+ tablets by Bonusan, also containing vitamin B12 and folic acid) was again deemed unsuitable.[20,22] The fourth supplement (20 mg capsules by Vitals) has been effective, but requires a current daily ingestion of 30 capsules.[23] Their administration provide difficult with physical resistance, forced swallowing and vomiting post-administration. Another treatment complication is transaminitis, which occurred after the initiation of PLP monotherapy (peak ASAT: 113 U/l, ref. 10–40, ALAT 115 U/l, ref: <45). This eventually prompted a reduction of the PLP dosage (from 40 to 31 mg/kg/d), with subsequent near normalization (most recent at age 6 ASAT: 59 U/l, ref. 10–40, ALAT 63 U/l, ref: <45).

3.3 Case 3.

This 14-month old male patient without a genetically confirmed diagnosis (despite trio whole exome sequencing) has been on PLP monotherapy (30 mg/kg/d) for clinically observed PLP-dependent seizures since he was 5 days old. This boy had been born as the first and only child to non-consanguineous Afghan parents. His birth was complicated by perinatal asphyxia. Twelve hours after his birth a neonatal epileptic encephalopathy became apparent. Treatment with anticonvulsive therapy included phenobarbital, levetiracetam and later phenytoin. Although his seizures proved intractable to these anticonvulsants, levetiracetam was continued. Continuous administration of midazolam iv was required. Pyridoxine was added on day 4 of life (first 15 mg/kg/d, one day later increased to 30 mg/kg/d, iv).

The occurrence of subclinical seizures persisted, his consciousness was severely impaired and subsequent analysis was inconsistent with pyridoxine-dependent epilepsy (Urine:

AASA 0.6 and 0.4 mmol/mol creatinine, ref. 0-2.0). Pyridoxine was discontinued a day later and a PLP trial (30/mg/d, 35,1 mg capsules by AOV, through nasogastric tube) was initiated.[22] Following this, the child became seizure free, his consciousness normalized within 2 days and midazolam could be discontinued successfully. The boy was discharged with PLP therapy (30 mg/kg/d) and levetiracetam (40mg/kg/d). During his hospitalization, repeat MRI's had shown widespread diffusion restriction and his head circumference faltered, features that were characterized as cerebral atrophy.

Subsequent genetic testing was performed and found no pathogenic variants in *PLPBP*. Therefore, PNPO enzyme activity was measured in a dried bloodspot and found to be normal (25.92 pmol/DBS/hr, ref. 10-90), suggesting this is not a case of PNPO deficiency. A heterozygous *PNPO* variant (c.347G>A; p.Arg116Gln) was detected, without a second pathogenic variant on the other allele.[27,28] Singleton exome sequencing was performed and showed no pathogenic variants deemed causal of the phenotype. Subsequent trio WES including the parent's DNA revealed no possibly causal variants. Therefore, the diagnosis or genetic defect underlying this case of his PLP-responsive epileptic encephalopathy has remained inconclusive; trio whole genome sequencing is currently pending.

At 3 months of age, a serious treatment complication occurred when the child was hospitalized with a PLP intoxication, which was discovered following his hospitalization with a severe respiratory insufficiency and bradypnea. One month prior, his PLP dosage had been increased, but following this intoxication it became apparent the dosage received (74 mg/kg/d) exceeded the intended dosage (50 mg/kg/d) due to unclear labelling of the PLP supplement (see table 2).[22] Biochemical analysis of the vitamin B6 vitamers revealed a significantly higher pyridoxal level than have previously been described in PLP therapy, although no reference values exist for this age (Plasma: PLP 351.1 nmol/l, pyridoxal 23657.0 nmol/l, pyridoxamine 280.0 nmol/l, pyridoxamine 5′-phosphate 5.1 nmol/l, pyridoxine 337.80 nmol/l pyridoxine 5′-

phosphate 4485.0 nmol/l). His transaminases were also elevated (ASAT: 126 U/l, ref. <89, ALAT 142 U/l, ref: <60). A hepatic ultrasound showed no abnormalities. The PLP dosage was subsequently tapered off (to 35mg/kg/d) and his clinical condition soon improved to his status pre-admission with severe global developmental delay, dyskinesia, microcephaly, visual disturbances and a feeding intolerance.

Following his discharge, his PLP dosage has been further reduced (31 mg/kg/d) but remains effective and no brand switches were required. At 8 months old, levetiracetam was also withdrawn successfully. At present, the patient ingests 7 dissolved capsules per day. His transaminases have normalized (most recent at age 8 months ASAT: 56 U/l, ref. <89, ALAT 45 U/l, ref: <60).

4. Discussion

We report three cases of PLP-dependent seizures wherein patients suffered complications secondary to food supplement quality issues occurring during the course of this treatment. In all three cases, the patients are critically dependent on PLP to maintain adequate seizure control. Thus, PLP functions as *de facto* antiepileptic medication, despite the lack of a licensed PLP product of pharmaceutical quality in the European Union. The only available and accessible form of PLP in the Netherlands, the UK and likely elsewhere are food supplements, which were never intended to be used for this purpose.[14] Since provisions regulating medication do not apply to food supplements, they are not produced in accordance with the same strict quality standards - including good manufacturing practice (GMP) guidelines, pre-market quality and safety reviews and post-market pharmacovigilance. Although in the Netherlands, there are specific regulations governing food supplements at the EU and national level, their stipulations are far less stringent.[29,30] Therefore, relying on food supplements as anti-seizure therapy is

worrisome. Previous analysis of PLP supplements justifies such concerns, demonstrating inconsistencies in PLP dose accuracy as well as weight inhomogeneity.[14]

The most serious complications we report are the occurrence of a status epilepticus due to PLP content variation and a PLP intoxication. The seizure following the introduction of a new PLP product in case 1 is thought to have been precipitated by variations in the actual PLP concentration of the prescribed supplement. The PLP intoxication that occurred in the third case was a direct result of unclear labelling of the product. The PLP supplement prescribed in this case was labelled as containing 35,1 mg PLP, but the same label stated a vitamin B6 content of only 20 mg.[22] The manufacturer has since changed the label, removing the higher PLP content, but it is unclear what this means for the PLP concentration in the product. This situation might have been prevented by the safeguards surrounding medicinal products, including pharmacovigilance systems and reference information resources.

Two additional factors deserve discussion: the need to dissolve tablets or capsules, with the risk of inactivation through light exposure, and the high amount of tablets or capsules. Light exposure accelerates degradation of the PLP, one of the hypothesized causes of hepatotoxicity and thus potentially aggravating the side effects associated with this therapy as well as reducing its effectivity.[14] Next, the ingestion of high volumes of medicines has resulted in emotional distress, vomiting and physical resistance – as illustrated in case 1 and 2. The 2018 legal limit on the PLP concentration in food supplements has aggravated this reality and precipitated switches to multiple brands of unknown quality and unverified PLP concentration, increasing the risk of triggering seizures or intoxication. The search for the most suitable supplement has proven arduous in case 1 and 2, requiring up to five brand switches per patient.

The situation described here is not unique to PLP-responsive seizures. In fact, similar situations occur in the treatment of other vitamin-responsive IMDs – at least 35 of which have been currently identified.[18] Vitamins are routinely prescribed to treat these disorders and

their effect can be of such crucial importance that they should be viewed as medicines. It is our belief that if a nutrient therapy's functioning is truly equivalent to medication in the context of an IMD, this product should also adhere to the same pharmaceutical quality standards as medication and should therefore be licensed and reimbursed as such. The treatment of PLP-dependent seizures may be the embodiment of the saying 'a vitamin a day keeps the doctor away'. However, for these patients – and perhaps for all patients with IMDs requiring similar treatments – we must add a crucial asterisk: not all vitamin products are suitable to keep the doctor away. In order to achieve this goal, there is a clear need for licensed nutrient products of a pharmaceutical quality for the treatment of IMDs.

Compliance with ethical standards

The present study was conducted in accordance with the Helsinki declaration. The retrospective review of medical files of the patients reported in this paper is conform the guidelines provided by our Institutional Review Boards.

Conflict of interest

The authors declare that they have no conflicts of interests.

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Table 1. *Overview of Diagnostics, PLP Treatment Description and Treatment Outcomes in 3 cases of patients treated with PLP*

Patient		***Diagnostics***		***Clinical***	***PLP Treatment***				
Case	***Current Age***	***PNPO* variant**	**CSF PLP (nmol/L)**	**Main symptoms, age at onset**	**Start of PLP (age)**	**PLP dose (mg/kg/d – #capsules/d)**	**PLP supplements (chronological)**	**Outcome PLP treatment**	**Hepatotoxicity (transaminases)**
1	*4y*	c.364-1G>A; p.(?)[4] + c.448_451del;p.ProArgfs*27[19](compound heterozygosity)	ND	Seizures, first hours of life	3 days	34 mg/kg/d- 33 capsules	Bonusan 50 mg AOV 20 mg Bonusan 20 mg** Vitals 20 mg Life Extension 100mg Vitals 20 mg	Adequate seizure control, developmental delay	Slightly elevated, normalizing following PLP dosage reduction
2	*6y*	c.481C>T; p.Arg161Cys[25] (homozygous)	21 (during pyridoxine therapy)	Seizures, first hours of life	17 months	31 mg/kg/d-30 capsules	Solgar 50 mg AOV 20 mg Bonusan 20 mg** Vitals 20 mg	Adequate seizure control, developmental delay	Elevated, slight elevation following PLP dosage reduction remains
3	*14mo*	c.347G>A; p.Arg116Gln[27,28](heterozygous variant); no second pathogenic variant. Normal PNPO enzyme activity*)	-	Seizures, 12 hours of life	5 days	31 mg/kg/d– 7 capsules	AOV 35,1 mg***	Adequate seizure control, severe developmental delay, cerebral atrophy	Elevated, normalizing following PLP dosage reduction

*ND: not detectable, *Measured in dried bloodspot; **This supplement also contains vitamin B12, active folate and folic acid; *** This supplement is labelled as containing 35,1 mg PLP, 20 mg of which is vitamin B6*

Table 2. *PLP/vitamin B6 food supplements prescribed to treat PLP-responsive epilepsy*

Brand, (country)	PLP content	Concentration verified	Form	Availability
Bonusan (NL)	50 mg	Yes, single batch tested[14]	Tablet	Discontinued[20]
	20 mg*	No	Tablet	Distributed in the Netherlands, Spain, Belgium, Austria and the UK, online availability for European consumers.
AOV (NL)	35,1 mg**	No	Capsule	Distributed in the Netherlands, Belgium and Germany, online availability in these markets through third-party sellers.[22]
Solgar (worldwide)	50 mg	Yes, single batch tested[14]	Capsule	Discontinued[26]
Vitals (NL)	20 mg	No	Capsule	Distributed in the Netherlands, online availability in the Netherlands and Belgium.[23]
Life Extension® (US)	100 mg	No	Capsule	Distributed in the US, online availability for international consumers[24], dosage exceeds Dutch legal limit[21]

*NL = Netherlands, US = United States; *This supplement also contains vitamin B12, active folate and folic acid, **This supplement was labelled as containing 35,1 mg PLP, 20 mg of which was vitamin B6. The manufacturer has since changed the label to only reflect a 20 mg vitamin B6 content.*

Highlights of our manuscript entitled "*A Vitamin a Day Keeps the Doctor Away: The Need for High Quality Pyridoxal-5'-Phosphate"*

- A rare subset of vitB6 responsive disorders requires PLP for seizure control.
- No licensed PLP product exists, so patients rely on poorly regulated food supplements.
- Serious complications occur as a result of supplement quality issues.
- Patients with other vitamin-responsive IMDs are at risk of similar complications.
- There is a clear need for licensed nutrient products of a pharmaceutical quality.

Conflict of interest statement

Conflict of interest

The authors declare that they have no conflicts of interests.

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