Brain & Development 41 (2019) 292–295

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case Report

Concentrations of various forms of vitamin B_6 in ginkgo seed poisoning

Tomomitsu Sado [a,*], Setsuko Nakata [a], Takahisa Tsuno [a], Masanori Sato [b], Yuka Misawa [c], Shoko Yamauchi [d], Yuji Inaba [d], Daisuke Kobayashi [e], Keiji Wada [e]

[a] Department of Pediatrics, Matsumoto Municipal Hospital, Japan
[b] Department of Pediatric Intensive Care, Nagano Children's Hospital, Japan
[c] Department of Rehabilitation, Nagano Children's Hospital, Japan
[d] Department of Pediatric Neurology, Nagano Children's Hospital, Japan
[e] Department of Food and Chemical Toxicology, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Japan

Received 8 June 2018; received in revised form 11 October 2018; accepted 16 October 2018

Abstract

A 2-year-old girl required medical attention for a sudden onset of repetitive tonic-clonic convulsions after ingesting 20–30 ginkgo seeds. Concentrations of the major forms of circulating vitamin B_6, pyridoxal-5′-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid, as well as the known ginkgo seed toxin 4′-*O*-methylpyridoxine (MPN) were measured in the serum and cerebrospinal fluid (CSF). PLP is an active form of vitamin B_6 and necessary for γ-aminobutyric acid (GABA) production. High MPN concentrations were observed in both the serum and CSF. As the PLP to PL ratio was markedly decreased in serum and CSF examinations, we suspected the ratio to be important in GABA production. This case report provides novel information on the metabolism of vitamin B_6 in humans as a result of ginkgo seed poisoning.
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Keywords: Ginkgo seed poisoning; Vitamin B_6; Pyridoxal-5′-phosphate; Pyridoxal; 4-Pyridoxic acid

1. Introduction

Ginkgo seeds are often eaten roasted or boiled in Asian countries. Especially in children, excessive ginkgo seed consumption can induce convulsions [1,2] by the 4′-*O*-methylpyridoxine (MPN) toxin [3]. Vitamin B_6 members related to ginkgo seed poisoning [1,4] include pyridoxal-5′-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (4-PA), which are the important forms of vitamin B_6 in the blood. Pyridoxal kinase is necessary for PL metabolization to the active form of vitamin B_6, PLP. Since MPN is thought to inhibit pyridoxal kinase, PLP may decrease and PL increase in ginkgo seed poisoning. PL metabolism to 4-PA leads to rise in 4-PA levels, with MPN believed to metabolize to PL as well [4] (Fig. 1).

PLP is a coenzyme in the glutamate decarboxylase (GAD) conversion of glutamate to γ-aminobutyric acid (GABA). Thus, a decrease in PLP presumably reduces GABA production. Vitamin B_6 injection of pyridoxal phosphate or pyridoxine is used to treat ginkgo seed poisoning by normalizing pyridoxal phosphate-dependent

* Corresponding author at: Department of Pediatrics, Matsumoto Municipal Hospital, 4417-180 Hata, Matsumoto, Nagano 390-1401, Japan.
E-mail address: t_sado@shinshu-u.ac.jp (T. Sado).

https://doi.org/10.1016/j.braindev.2018.10.007
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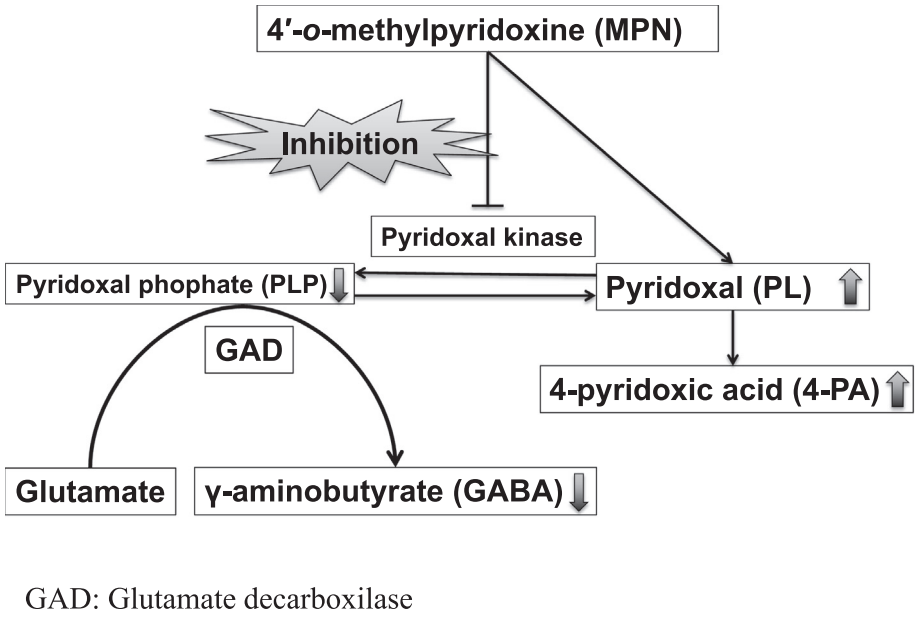

Fig. 1. In ginkgo seed poisoning, it is thought that PL and 4-PA increase and PLP decreases, leading to a GABA production decrease [4].

enzymes in the brain and restoring normal GABA production [4–6].

We recently encountered a 2-year-old girl who suffered from acute ginkgo seed poisoning. This is the first report evaluating the concentrations of PLP, PL, 4-PA, and MPN in both the serum and cerebrospinal fluid (CSF) in such a case.

2. Case

A 2-year-old girl presented with the sudden onset of tonic-clonic convulsions and was transported to our hospital. She had no growth or developmental abnormalities and no remarkable medical history. It was revealed that the patient ingested 20–30 ginkgo seeds approximately 1 h before her convulsions began and the same amount 1 day earlier (Fig. 2). Her 12-year-old brother had also eaten roughly the same amounts but did not exhibit such symptoms. Apart from slight drowsiness, the patient's general condition was good. Physical examination revealed no specific abnormalities. Blood examination and head computed tomography were normal. Three hours after ingesting the ginkgo nuts, the patient suffered repeated convulsions that spontaneously resolved. Intravenous lactated Ringer's solution was administered. Due to the relapse she was transported to an intensive care unit at a tertiary emergency hospital and underwent a second blood test along with CSF examination, electroencephalogram, and head magnetic resonance. No abnormalities were noted. At 6 h post-ingestion, a third bout of convulsions occurred that spontaneously resolved. No further seizure or abnormalities in consciousness were observed after intravenous injection of 10 mg/kg pyridoxal phosphate. The patient was discharged 3 days later and has not exhibited further problems.

During the patient's clinical course, PLP, PL, 4-PA, and MPN concentrations in the serum and CSF were evaluated using high-performance liquid chromatography [4]. High concentrations of MPN were detected in both the serum and CSF. As serum MPN concentrations decreased gradually, serum PL and 4-PA decreased and serum PLP increased. Serum PLP to PL ratio increased over time (Tables A and B). The patient's family provided written consent to measure the concentrations of MPN, PLP, PL, and 4-PA and publish the clinical features of the case. This study was reviewed and approved by the ethics committee of our institution.

3. Discussion

This case report describes the metabolism of vitamin B_6 in humans following ginkgo seed poisoning. The patient's serum concentrations of MPN, PLP, PL, and 4-PA changed predictably over the course of treatment. In an earlier study, the administration of MPN decreased PLP concentration and increased PL and 4-PA concentrations in rat plasma [4]. Ginkgo seed poisoning also led to a serum PLP decrease in an adult case [1]. As there were no reference PLP, PL, or 4-PA level ranges for children available, we compared the patient's findings with those of 1–6-year-old children with neurological symptoms [7], in whom both serum and CSF concentrations of PL and 4-PA were elevated. There was no apparent decrease in PLP in our subject, but her PLP to PL ratio indicating the balance of vitamin

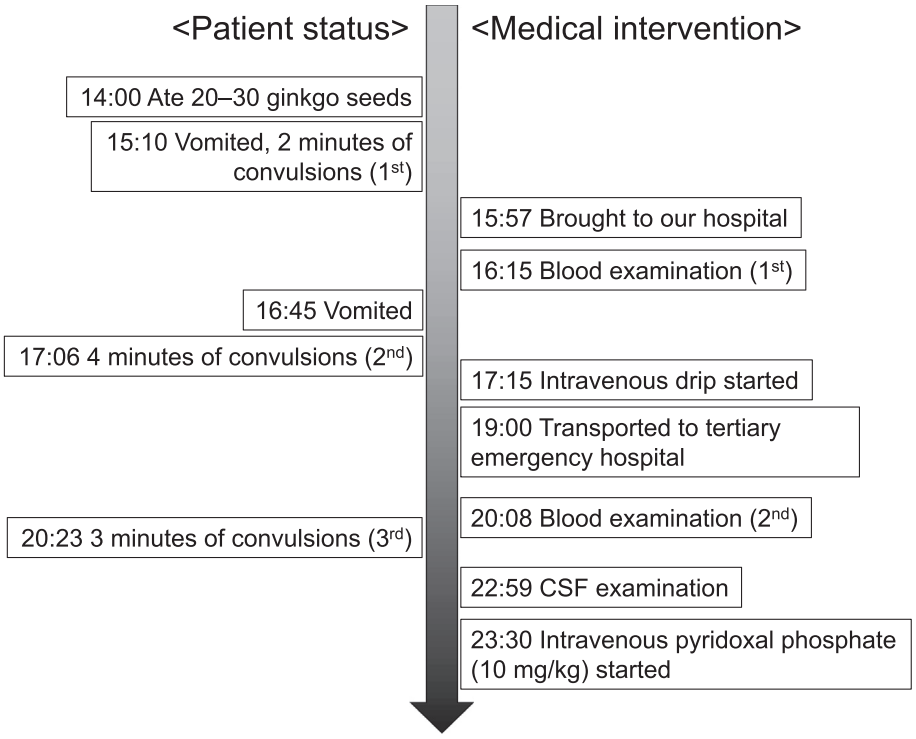

Fig. 2. Patient progress after ingesting Ginkgo seeds.

Table A
Concentrations of PLP, PL, 4-PA, and MPN in serum and CSF.

	PLP (nmol/L)	PL (nmol/L)	4-PA (nmol/L)	MPN (nmol/L)	PLP/PL
Serum (2 h after ingestion)	44	308	233	2752	0.14
Serum (6 h after ingestion)	51	100	93	1356	0.51
CSF (9 h after ingestion)	37	521	8	1360	0.07

The concentrations of PLP, PL, 4-PA and MPN in the serum and CSF of the patient. Table B presents the range (median) of PLP, PL, and 4-PA concentrations in the serum and CSF obtained from 1 to 6-year-old children with neurological symptoms [7].

Table B
Representative range and median PLP, PL and 4-PA concentrations in serum and CSF [7].

	PLP (nmol/L)	PL (nmol/L)	4-PA (nmol/L)	PLP/PL
Serum	16.2–57.4 (34.7)	8.8–28.0 (14.5)	6.4–26.4 (10.7)	1.9–5.3 (2.9)
CSF	7.8–31.5 (20.1)	16.5–41.1 (26.1)	<1.2[a]–1.5 (<1.2[a])	0.6–2.0 (1.1)

[a] Below the limit of quantification (1.2 nmol/L).

B_6 phosphorylation/dephosphorylation in the body [7] was severely repressed (Tables A and B). We suspected that MPN was metabolized to excess PL, which was further metabolized to PLP.

A limitation of this report was that we did not measure intraneuronal PLP concentrations in the brain since the amounts of PLP in the serum and CSF did not indicate a decrease in GABA production. On the other hand, there was an obvious decline in PLP to PL ratio. This ratio decreases when alkaline phosphatase (ALP) activity has increased or pyridoxal kinase activity has decreased [4,8]. The patient's ALP level was 520 IU/L at our hospital and not indicative of elevated activity (normal range: 410–1150 IU/L [9]). Thus, her decrease in PLP to PL ratio suggested a fall in pyridoxal kinase activity and presumably reflected a decrease in GABA production.

Lastly, it was also possible that phosphorylated MPN suppressed the activity of GAD directly. Two isoforms of GAD, GAD_{65kDa} and GAD_{67kDa}, exist in the human brain, and high concentrations of 4′-*O*-methylpyridoxine-5′-phosphate (MPNP) have been shown to decrease GAD_{65kDa} activity. In a previous report, in vitro GAD_{65kDa} activity was reduced to 50% in the presence

of 2.7 mM MPNP [5,10]. Our patient's MPNP was undetectable in the serum and only 31 nM in the CSF. As these amounts were too small to suppress the activity of GAD directly, direct GAD suppression was considered unlikely.

This is the first report examining the concentrations of PLP, PL, and 4-PA in both the serum and CSF in a case of ginkgo seed poisoning that also considered the importance of PLP to PL ratio. Additional case data are needed to characterize the changes in PLP, PL, and 4-PA in ginkgo seed poisoning, which may shed light on the relationship between PLP and the other forms of vitamin B_6.

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