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Communication

# Decrease in pyridoxal-5′-phosphate concentration and increase in pyridoxal concentration in rat plasma by 4′-O-methylpyridoxine administration

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ABSTRACT

Food poisoning from *Ginkgo biloba* seeds can cause epilepsy because of a decrease in γ-aminobutyric acid (GABA) concentrations in the brain. We previously demonstrated that 4′-O-methylpyridoxine (MPN) is responsible for this observed toxicity of *G biloba* seeds; however, the mechanism for the decrease in GABA and plasma concentration profile of MPN has not been clarified. Our hypothesis is that MPN induces a decrease in vitamin $B_6$ concentrations, resulting in a decrease in GABA concentration. This study aimed to characterize the plasma concentration profile of MPN and intrinsic vitamin $B_6$ concentrations (pyridoxal [PL], PL-5′-phosphate [PLP], and 4-pyridoxic acid) using a rat model. Plasma concentrations of $B_6$ vitamers after intravenous MPN administration (5 mg/kg) were determined using high-performance liquid chromatography with a fluorescence detector. The half-life of MPN (0.91 ± 0.05 hours) was shorter in rats than the previously reported value in humans. We found a significant decrease in the plasma concentration of PLP, an active form of vitamin $B_6$, after MPN administration. We also observed an increase in plasma PL and 4-pyridoxic acid concentrations; the increase in PL concentration may be caused by either metabolism of MPN to PL or by MPN-mediated inhibition of PL kinase. The present study is the first *in vivo* study showing relatively rapid elimination of MPN in rats and a decrease in plasma PLP concentration caused by MPN.



## 1. Introduction

Vitamin $B_6$ is an important nutrient involved in many enzymatic reactions including amino acid metabolism [1]. Vitamin $B_6$ occurs in several forms, which can be interconverted. The main forms of vitamin $B_6$ in plasma are pyridoxal-5′-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (4-PA) [2–5]. Pyridoxal-5′-phosphate is an active form of vitamin $B_6$, and the conversion between PLP and PL is regulated by PL kinase and alkaline phosphatase [6]. 4-Pyridoxic acid, which is produced from PL, is the end metabolite of vitamin $B_6$ and excreted in urine [7,8].

*Abbreviations:* GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; MPN, 4′-O-methylpyridoxine; MPNP, 4′-O-methylpyridoxine-5′-phosphate; 4-PA, 4-pyridoxic acid; PL, pyridoxal; PLP, pyridoxal-5′-phosphate; PN, pyridoxine.

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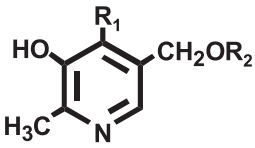


| | $R_1$ | $R_2$ |
|---|---|---|
| PN | $CH_2OH$ | H |
| PL | CHO | H |
| PLP | CHO | $PO_3H_2$ |
| 4-PA | COOH | H |
| MPN | $CH_2OCH_3$ | H |
| MPNP | $CH_2OCH_3$ | $PO_3H_2$ |

**Fig. 1 – Structures of vitamin $B_6$ analogues. Structures of PN, PL, PLP, 4-PA, MPN, and MPNP are shown.**

Overconsumption of *Ginkgo biloba* seeds can lead to tonic and/or clonic convulsions, vomiting, and loss of consciousness [9–17]. We previously demonstrated that 4′-*O*-methylpyridoxine (MPN; 3-hydroxy-5-hydroxy-methyl-4-methoxymethyl-2-methylpyridine, or ginkgotoxin; Fig. 1) and MPN-5′-glucoside are responsible for food poisoning by *G biloba* seeds [18–20], which are called *Ginnan* in Japan and consumed in China, Korea, and Japan. More than 100 cases of food poisoning by *G biloba* seeds have been reported in Japan until 2005 [15].

4′-*O*-methylpyridoxine, an antivitamin $B_6$ compound, causes epilepsy by decreasing the level of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, in the brain [21–23]. However, the mechanism underlying the decrease in GABA concentration by MPN has been controversial. One hypothesis is that MPN directly inhibits glutamate decarboxylase (GAD) [21], which produces GABA from the excitatory neurotransmitter glutamate. Another hypothesis is that MPN inhibits PL kinase [23–25], an important enzyme for active vitamin $B_6$ (PLP) production. This latter hypothesis has been more readily accepted because *in vitro* studies have shown that the inhibitor affinity of MPN against PL kinase was higher than that against GAD [25,26]. However, the *in vivo* role of these enzymes during *G biloba* seed poisoning remains unclear.

To date, vitamin $B_6$ levels in plasma after *G biloba* seed or MPN consumption have not been reported. In addition, the *in vivo* plasma concentration profile of MPN after its administration is not known, although previous reports indicate the presence of MPN in the serum of patients with *G biloba* seed poisoning [9,14,16,17]. Our hypothesis is that MPN decreases vitamin $B_6$ concentration *in vivo*, thereby inducing vitamin $B_6$–deficient symptoms. Therefore, in this study, the effects of MPN on intrinsic vitamin $B_6$ concentration and plasma concentration profile of MPN were examined.

## 2. Methods and materials

### 2.1. Chemicals

Pyridoxal hydrochloride, PLP hydrochloride, and 4-PA were purchased from Sigma (St Louis, MO, USA). Pyridoxine (PN) hydrochloride was obtained from Wako Pure Chemical (Osaka, Japan). 4′-*O*-methylpyridoxine and MPN-phosphate (MPNP) were chemically synthesized, as reported previously [24,27]. All other reagents were of analytical grade.

### 2.2. Chromatographic conditions

High-performance liquid chromatography (HPLC) conditions were optimized from a previously reported method [3]. The HPLC apparatus comprised a Shimadzu LC-10Avp system equipped with an RF-10A spectrofluorometer (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on an Inertsil ODS-3 column (150 × 4.6 mm in id, 5 μm; GL Sciences, Tokyo, Japan) at a flow rate of 1.0 mL/min. The column temperature was ambient. Mobile phase A was 60 mM disodium hydrogen phosphate containing 400 mg/L EDTA disodium salt, adjusted to pH 6.5 with concentrated phosphoric acid; mobile phase B was methanol. The ratio of mobile phases A to B was 90:10. For determining concentrations of vitamin $B_6$ analogues (except aldehyde types of vitamin $B_6$, PL, and PLP), fluorescence measurement was performed at 420-nm emission wavelength with 320-nm excitation wavelength. For the identification of PL and PLP, fluorescence measurement was performed at 450-nm emission wavelength, with 380-nm excitation wavelength after derivatization by semicarbazide [3].

### 2.3. Animals

Eight-week-old male Wistar-ST rats were obtained from Sankyo Labo Service Co (Tokyo, Japan). All rats had free access to food and water. Before the experiment, rats were fasted overnight with free access to water. All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in Health Sciences University of Hokkaido.

### 2.4. Intravenous MPN administration to rats

4′-*O*-methylpyridoxine was dissolved in saline (3.33 mg/mL). Rats were anesthetized with intraperitoneal injection of urethane (1 g/kg). 4′-*O*-methylpyridoxine solution (1.5 mL/kg body weight = 5 mg/kg body weight) or saline was administered into either the right or left jugular vein of rat. Blood was periodically collected from the other side of the jugular vein before and after the intravenous administration of MPN or saline to rats. Plasma was separated from blood by centrifugation for 10 minutes (2500 × g).

### 2.5. Sample preparation

Rat plasma was diluted to a total of 130 μL with distilled water. The sample was pre-column derivatized using a 10-μL derivatization agent, containing 250 mg/mL semicarbazide hydrochloride and 250 mg/mL glycine for 30 minutes at room temperature in dark [3]. After derivatization, 10 μL of 70% perchloric acid was added for deproteinization, and the sample was centrifuged for 10 minutes (9000 × g). The supernatant was then transferred to a new microcentrifuge tube, neutralized by KOH addition, and centrifuged for 10 minutes (9000 × g). The filtered supernatant (10 μL) was injected into the HPLC apparatus.

### 2.6. Kinetic analysis

The elimination half-life ($t_{1/2}$), elimination rate constant ($k_{el}$), mean residence time, total clearance, and steady-state distribution