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Negligible Effect of Oral Garlic Oil on the Oral Absorption of Pyridoxine in Metadoxine in Rats

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Metadoxine [an ion-pair between pyridoxine and pyrrolidone carboxylate (PCA)] plus garlic oil treatment synergistically reduces alcoholic steatosis compared to each agent alone. We evaluated the effect of garlic oil on the pharmacokinetics of pyridoxine. After the oral administration of metadoxine, the total area under the plasma concentration-time curve from time zero to time infinity (AUC) and the peak plasma concentration (C_{max}) of pyridoxine were significantly greater (by 40.6%) and higher (by 63.9%), respectively, than after oral administration of pyridoxine plus PCA. Oral metadoxine plus garlic oil also gave larger AUC (31.8%) and higher C_{max} (64.9%) than pyridoxine plus PCA. However, garlic oil did not change the AUC or C_{max} of pyridoxine in metadoxine. Thus, garlic oil does not enhance the metadoxine activity by affecting the absorption of pyridoxine.

Key words: Metadoxine with and without garlic oil, Pyridoxine plus PCA, Pharmacokinetics, Rats

INTRODUCTION

Both pyridoxine (vitamin B6) and pyrrolidone carboxylate (PCA; an intermediate in the synthesis/degradation of glutathione) are safe and naturally occurring substances. Metadoxine is pyridoxol l-2-pyrrolidone-5-carboxylate, which is the ion-pair between pyridoxine and PCA. Metadoxine is useful for the treatment of alcoholics and alcoholic liver disease (Santoni et al., 1989; Bono et al., 1991; Arosio et al., 1993; Rizzo et al., 1993). Metadoxine treatment increases the clearance of alcohol and acetaldehyde, reducing the damaging effect of free radicals and restoring cellular adenosine triphosphate (ATP) and glutathione levels (Caballería et al., 1998; Calabrese et al., 1998; Gutiérrez-Ruiz et al., 2001). Pyridoxine is absorbed from the gastrointestinal tract by an efficient, specialized, carrier-mediated mechanism dependent on acidic extracellular pH (Said and Mohammed, 2006). Pyridoxine is primarily metabolized in the liver to pyridoxal 5'-phosphate and oxidized to the deadend catabolite 4-pyridoxic acid (Merrill and Henderson, 1990).

The organosulfur ingredients in garlic oil (GO) exert chemoprotective effects against chemical-induced carcinogenesis in rats (Brady et al., 1988) by inducing phase II detoxifying enzymes (Hayes et al., 1987) and inhibiting hepatic cytochrome P450 (CYP) 2E1 induction, which produces reactive metabolic intermediates from a wide variety of organic molecules (Brady et al., 1991). Recently, Ki et al. (2007) reported that combined metadoxine and GO treatment reduced alcoholic steatosis and CYP2E1 induction in rat liver after receiving an alcohol-fed diet for 4 weeks. The combination of metadoxine and GO also synergistically inhibits alcohol-induced hepatic triglyceride accumulation and improves liver function compared to individual agents alone. However, the pharmacokinetic interaction between metadoxine and GO is unclear. The pharmacokinetics of intravenous metadoxine in humans has been reported (Lü et al., 2006, 2007), but no studies in rats.

We did not observe a metadoxine peak with LC-MS/MS using a 10 μg/mL metadoxine solution in water

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[mobile phase, 10 mM ammonium formate : acetonitrile = 5 : 5 (v/v); column, Atlantis dC_{18} (2.1 mm, *l.* × 100 mm, i.d.; particle size, 3 μm)] at 30°C, and only pyridoxine and PCA peaks were observed [the (M+H) + peaks, m/z = 299, 170, and 130 for metadoxine, pyridoxine, and PCA, respectively], indicating that the ion-pair in metadoxine is rapidly broken to form pyridoxine and PCA, making metadoxine analysis impossible.

The purpose of this study was to evaluate the possible effect of oral GO on the pharmacokinetics of oral pyridoxine in metadoxine in rats.

MATERIALS AND METHODS

Chemicals

Metadoxine and GO were donated from Pharma-King Pharmaceutical Company. Pyridoxine HCl and PCA were purchased from Sigma-Aldrich Corporation. Other chemicals were of reagent or high-performance liquid chromatographic (HPLC) grade.

Animals

Protocols for this animal study were approved by the Animal Care and Use Committee of College of Pharmacy, Seoul National University, Seoul, Republic of Korea. Male Sprague-Dawley rats (6-9 weeks old, weighing 270-315 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea). They were maintained in a clean-room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at 20-23°C with 12-h light (07:00-19:00 h) dark cycles and a relative humidity of 50 ± 10%. The rats were housed in metabolic cages (Tecniplast) under filtered pathogen-free air and with food (Samyang Company) and water available *ad libitum*.

Oral administration of pyridoxine plus PCA, metadoxine, and metadoxine with GO

The procedures used for pretreatments of rats, including cannulation (early in the morning) of the carotid artery (for blood sampling), were similar to a reported method (Lee et al., 2006). Rats were lightly anesthetized with ether and the experiment was started 4-5 h after surgery. Rats were randomly divided into three oral groups: pyridoxine HCl (114 mg/kg) plus PCA (86 mg/kg; both dissolved in distilled water) (n = 7), 200 mg/kg metadoxine (dissolved in distilled water), which is equivalent to 114 mg/kg of pyridoxine with 86 mg/kg of PCA (n = 9), and 200 mg/kg metadoxine with GO (0.2 mL/kg) (n = 7). The total oral volume was 5 mL/kg using a gastric gavage tube. Blood samples (approximately 0.22 mL, each) were collected via the carotid artery at 0 (control), 5, 15, 30, 60, 90, 120, 240, 360, 480, 600, and 720 min after oral administration of the drug (s). A heparinized 0.9% NaCl-injectable solution (15 units/mL), 0.3 mL, was used to flush the cannula immediately after each blood sampling to prevent blood clotting. Each blood sample was immediately centrifuged and 50-μL was collected in a 1.5 mL polyethylene tube and stored at −70°C (Revco ULT 1490 D-N-S; Western Mednics) until HPLC analysis of pyridoxine. The procedures used for collecting and handling of the gastrointestinal tract (including its contents and feces) at 24 h ($GI_{24\,h}$) were similar to a reported method (Lee et al., 2006).

HPLC analysis of pyridoxine

Acetonitrile (150-μL) was added to deproteinize 100 μL samples. After vortex-mixing and centrifugation (13,100 rpm, 2 min), 50 μL of the supernatant was directly injected onto a reverse-phase (C_{18}) HPLC column (3.9 mm, *l.* × 150 mm, i.d.; particle size, 5 μm; Waters). The ratio of the mobile phase A to B (mobile phase A, 20 mM KH_2PO_4 buffer, pH 3.0, 100%; mobile phase B, acetonitrile, 100%) was 100% up to 4 min after running and gradually changed to 40% up to 9 min after running at a flow-rate of 1 mL/min. The column eluent was monitored using a fluorescence detector (TSP) at an excitation wavelength of 286 nm and an emission wavelength of 400 nm, at room temperature. The retention time of pyridoxine was approximately 3.3 min, and detection limits of pyridoxine in the plasma and gastrointestinal samples were all 50 ng/mL. Coefficients of variation were below 7.36%.

Pharmacokinetic analysis

The total area under the plasma concentration-time curve from time zero to infinity (AUC) was calculated using the trapezoidal rule-extrapolation method (Chiou, 1978). The area from the last datum point to infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant. The terminal half-life was calculated by dividing 0.693 by the terminal-phase rate constant. The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data.

Statistical analysis

$P < 0.05$ was considered statistically significant using a Duncan's multiple range test in SPSS with *posteriori* analysis of variance (ANOVA) among the three sets of unpaired data. All data are expressed as mean ± S.D.

RESULTS AND DISCUSSION

In rats pretreated with a normal diet containing 7 mg/kg pyridoxine HCl for 6 weeks, the plasma concentration of pyridoxine was very low, approximately 5.08 ng/mL (Schaeffer et al., 1989), compared with a detection limit of 50 ng/mL. Therefore, basal pyridoxine levels in plasma should not affect our pyridoxine study. GO (0.2 mL/kg) was used based on efficacy results in alcoholic steatosis by combined treatment of metadoxine and GO (50 or 100 mg/kg) for 6 consecutive days (Ki et al., 2007).

The mean arterial plasma concentration-time profiles of pyridoxine after the oral administration of pyridoxine HCl plus PCA, metadoxine, and metadoxine with GO are shown in Fig. 1 and pharmacokinetic parameters are listed in Table I. Absorption of pyridoxine after oral administration of pyridoxine HCl plus PCA, metadoxine, and metadoxine with GO groups was rapid; the T_{max}s were 57.9, 20.0, and 32.1 min, respectively. The absorption of pyridoxine after metadoxine administration was greater than that after pyridoxine HCl plus PCA; the AUC was larger (40.6%) and the C_{max} higher (63.9%) with metadoxine than with pyridoxine HCl plus PCA, suggesting that ion-pairing between pyridoxine and PCA in metadoxine could enhance pyridoxine absorption (possibly due to the decrease in polarity and increase in permeability of pyridoxine). Thus, metadoxine has better pharmacokinetics than pyridoxine plus PCA. The AUC of pyridoxine was greater (31.8%) and C_{max} higher (64.9%) after oral metadoxine with GO than after oral pyridoxine HCl plus PCA. The $GI_{24\,h}$ (including GI content and feces) of pyridoxine were negligible; less than 0.551% of the oral dose. Metadoxine alone and with GO produced similar changes in pyridoxine pharmacokinetics, although GO improved the therapeutic effect of metadoxine in rats (Ki et al., 2007). The diallylsulfide in garlic affects hepatic CYPs and P-glycoprotein (P-gp) (Foster et al, 2001; Arora et al., 2004; Davenport and Wargovich, 2005). It is unclear whether pyridoxine is a substrate of CYPs or P-gp, but regardless, GO did not influence pyridoxine pharmacokinetics after coadministration with metadoxine, indicating that the pharmacological synergism between metadoxine and GO does not result from a pharmacokinetic interaction. Combination of metadoxine and GO may reduce the fat accumulation and CYP2E1 induction and inhibit lipid accumulation based on histopathology of the liver and blood chemistry data (Ki et al., 2007). The decrease in AMP-activated protein kinase-α (AMPKα) phosphorylation was restored by metadoxine and GO, potentially indicating increased in acetyl-CoA carboxylase phosphorylation (Ki et al., 2007). However, we could not exclude the possibility that long-term GO administration may affect metabolic enzymes related to pyridoxine metabolism.

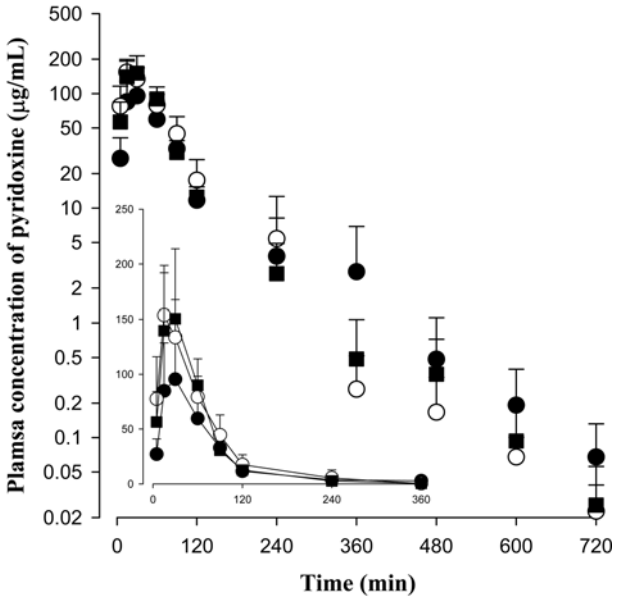

Fig. 1. Mean arterial plasma concentration-time profiles of pyridoxine after the oral administration of pyridoxine (114 mg/kg) plus PCA (86 mg/kg), (●; n = 7), metadoxine (200 mg/kg), (○; n = 9), or metadoxine (200 mg/kg) with garlic oil (■; n = 7) to rats. The inset shows the profile in a rectilinear graph up to 360 min. Bar represents the standard deviation.

Table I. Mean (± S.D.) pharmacokinetic parameters of pyridoxine after the oral administration of pyridoxine (114 mg/kg) plus PCA (86 mg/kg), metadoxine (200 mg/kg), and metadoxine (200 mg/kg) with garlic oil (0.2 mL/kg)

Parameter	Pyridoxine plus PCA (n = 7)	Metadoxine (n = 9)	Metadoxine with garlic oil (n = 7)
Body weight (g)	279 ± 7.87	289 ± 14.3	290 ± 16.7
AUC (μg min/mL)	7540 ± 1950[a]	10600 ± 1680	9940 ± 2090
C_{max} (μg/mL)	97.0 ± 47.3[a]	159 ± 44.1	160 ± 49.6
T_{max} (min)	57.9 ± 80.5	20.0 ± 7.50	32.1 ± 13.5
Terminal half-life (min)	53.6 ± 7.76	56.2 ± 32.5	52.9 ± 14.5
$GI_{24\,h}$ (% of the dose)	0.521 ± 1.12	0.548 ± 1.24	0.551 ± 0.962

[a] Pyridoxine plus PCA group was significantly different ($p < 0.05$) from metadoxine and metadoxine with garlic oil groups.

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