

EFFECTS OF CVT-E002, A PROPRIETARY EXTRACT FROM THE NORTH AMERICAN GINSENG (*Panax quinquefolium*) ON HEPATIC DRUG-METABOLIZING ENZYMES IN C57BL/6J MICE

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CVT-E002, a proprietary extract from North American ginseng (*Panax quinquefolium*) showed immunomodulating activity. Cytochrome P450 (CYP), UDP-glucuronosyl transferase (UGT), and glutathione S-transferase (GST) are important drug-metabolizing enzymes. Modulation of drug-metabolizing enzymes is a main cause of drug interactions. To assess the possible metabolism-based drug interaction of CVT-E002, effects of CVT-E002 on hepatic CYP, UGT, and GST were studied in C57BL/6J mice. Treatment of mice with 5g/kg/day CVT-E002 for three days had no effects on liver microsomal CYP and cytochrome *b*₅ contents and NADPH-CYP reductase activity. CVT-E002 had no effect on microsomal CYP catalytic activities of the oxidations of 7-ethoxyresorufin, 7-methoxyresorufin, benzo(a)pyrene, 7-ethoxycoumarin, benzphetamine, N-nitrosodimethylamine, erythromycin, and nifedipine in mouse liver. Hepatic microsomal UGT and cytosolic GST activities were not affected by CVT-E002-treatment. These results suggested that CVT-E002 had no effects on hepatic CYP, UGT, and GST activities in mice under this treatment regimen. There might be low potential of incidence of metabolism-based drug interaction by CVT-E002.

Key words: CVT-E002, Cytochrome P450, UDP-Glucuronosyl transferase, Glutathione S-transferase.

INTRODUCTION

Modulation of drug-metabolizing enzymes can change the plasma concentrations of drugs and result in serious drug interactions in humans. Phase I and phase II drug-metabolizing enzymes play important roles in the determination of pharmacological and toxicological effects of drugs¹. Cytochrome P450 (CYP)-dependent

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monooxygenase is the main phase I enzymes catalyzing oxidative metabolism of drugs. CYP-catalyzed oxidations require electron transfer through NADPH-CYP reductase. For some CYP enzymes, cytochrome b_5 is essential for the optimal catalytic activity². A series of evidence showed that alteration of the CYP pool could affect the biological effects of xenobiotics possibly due to the broad substrate specificities of CYP enzymes^{3,4}. Microsomal UDP-glucuronosyl transferase (UGT) and cytosolic glutathione S-transferase (GST) are the main phase II enzymes. UGT catalyzes the formation of glucuronide-conjugates of many endogenous and exogenous substrates including bilirubin and drugs. Glucuronides of drugs may accumulate during long term therapy and cause toxicity⁵. GST plays an important role in the detoxication of xenobiotics. CYP, UGT, and GST are responsive to the inductive and inhibitory effects of many exogenous factors including exposure to medicinal herbs, edible plants, environmental pollutants and drugs⁶.

Polysaccharides isolated from the North American ginseng (*Panax quinquefolium*) have been reported to have immunomodulatory effects⁷. CVT-E002 is a commercially available aqueous extract of North American ginseng with chemically and functionally consistent properties. The main constituents of CVT-E002 are polysaccharides. CVT-E002 stimulated *in vivo* immunoglobulin G production in mice⁸. It has been proven to show preventive effect against viral infection in a double-blinded placebo-controlled phase II clinical trial⁹. The increase in concurrent treatment of herbs and drugs increases the potential incidence of drug interactions. However, there is no report providing information pertaining to drug interaction for this commercially available natural product. Thus, we have studied the effect of CVT-E002 on drug-metabolizing enzymes. In the present report, our results showed that treatment of 5 g/kg/day CVT-E002 for 3 days had no effect on hepatic CYP, UGT, and GST activities in mice.

MATERIALS AND METHODS

Materials

CVT-E002 was obtained from CV Technologies Inc. (Edmonton, Canada). Benzo(a)pyrene, cytochrome *c*, 7-ethoxyresorufin, 7-methoxyresorufin, NADH, NADPH, and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Pyridine metabolite of nifedipine was a gift from Dr. F. Peter Guengerich (Nashville, TN, U.S.A.).

Animal treatment and microsomal preparation

Male C57BL/6J mice (5 weeks old, weighing 13 ~ 16g) were purchased from the National Laboratory Animal Breeding and Research Center in Taiwan. Before experimentation, mice were allowed a one-week acclimation period in the animal quarters with air conditioning ($25 \pm 1^\circ\text{C}$) and an automatically controlled photoperiod of 12 hr light daily. All experimental protocols involving animals were reviewed and approved by

the Institutional Animal Experimentation Committee of the National Research Institute of Chinese Medicine. Mice were given water and laboratory rodent chow (#5P14, PMI Feeds Inc., Richmond, IN, U. S. A.) *ad libitum* throughout the entire experiment. CVT-E002 was suspended in deionized water. Mice were treated with 5 g/kg/day CVT-E002 by gastrogavage for three days. Mice in the control group received same volumes of deionized water *per* kg body weight. Livers were removed and washed microsomes were prepared by differential centrifugation 22 hr after the last treatment¹⁰.

Monooxygenase assays

CYP and cytochrome *b*₅ contents were determined following the method of Omura and Sato¹¹. NADPH-CYP reductase activity was determined following the method of Phillips and Langdon¹² using cytochrome *c* as a substrate. In benzo(a)pyrene hydroxylation (AHH) assay, the formation of phenolic metabolite was determined using 3-hydroxybenzo(a)pyrene as a standard¹³. The O-dealkylations of 7-ethoxyresorufin and 7-methoxyresorufin were determined by measuring fluorescence of resorufin¹⁴. 7-Ethoxycoumarin O-deethylation (ECOD) was determined by measuring the fluorescence of hydroxycoumarin¹⁵. Nifedipine oxidation was determined following the method of Guengerich *et al.*¹⁶. N-Demethylations of benzphetamine, erythromycin, and N-nitrosodimethylamine were determined by measuring the formation of formaldehyde using Nash's reagent¹⁷. Microsomal UDP-glucuronosyl transferase (UGT) activity was assayed following the method of Bock *et al.*¹⁸ using *p*-nitrophenol as a substrate. Cytosolic glutathione S-transferase (GST) activity was determined using 1-chloro-2,4-dinitrobenzene as a substrate in the presence of glutathione¹⁹. Microsomal and cytosolic protein concentrations were determined by the method of Lowry *et al.*²⁰.

Statistical analysis

Statistical analyses of the differences between control and CVT-E002-treated groups were evaluated by the Student's t-test. A $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Our results showed that treatment of C57BL/6J mice with 5 g /kg/day CVT-E002 for three days had no effects on microsomal CYP and cytochrome *b*₅ contents and NADPH-CYP reductase activity in the liver (Table 1). It also had no effects on CYP-catalyzed oxidations of benzo(a)pyrene, 7-ethoxyresorufin, 7-methoxyresorufin, benzphetamine, 7-ethoxycoumarin, N-nitrosodimethylamine, erythromycin, and nifedipine (Table 2). As the family of CYP has broad substrate specificities, selective substrates can be used as chemical probes for CYP enzymes²¹. Benzo(a)pyrene could be metabolized by several CYP enzymes and CYP1A showed the highest AHH activity²². 7-Ethoxyresorufin O-deethylation (EROD) and 7-methoxyresorufin O-demethylation (MROD) are markers of reactions catalyzed by CYP1A1/2^{23,24}. Benzphetamine N-

demethylation (BDM) is mainly catalyzed by CYP2B1 in the rat²⁵. ECOD appears to be catalyzed principally by human CYP2E1²⁶. The demethylation of N-nitrosodimethylamine (NDM) is mainly proceeded by CYP2E1, which is in an ethanol inducible form in rodents²⁷. Erythromycin and nifedipine are marker substrates for CYP3A in mammals¹⁶. These results suggested that at the dose used CVT-E002 had no interactions with the substrates of CYP1A, CYP2B, CYP2E1, and CYP3A. For phase II enzymes, our results showed that CVT-E002 had no effects on hepatic UGT and GST activities in the liver of C57BL/6J mice (Table 3).

Table 1. Effects of CVT-E002 on body and liver weights and monooxygenase component contents and activity in mouse liver

	Control	CVT-E002
Body weight g	19.9 ± 0.26	21.4 ± 0.5
Liver weight g	0.93 ± 0.06	1.07 ± 0.08
Liver weight/body weight g/g	0.05 ± 0.00	0.05 ± 0.00
Cytochrome P450 nmol/mg protein	0.66 ± 0.04	0.69 ± 0.13
Cytochrome <i>b</i> ₅ nmol/mg protein	0.32 ± 0.01	0.38 ± 0.04
NADPH-Cytochrome P450 reductase nmol/min/mg protein	245.5 ± 24.3	304.5 ± 35.8

Mice were treated with 5 g/kg/day CVT-E002 suspended in water by gastrogavage for three days. Results represent means ± SE of six and five mice in control and CVT-E002-treated groups, respectively.

Table 2. Effects of CVT-E002 on monooxygenase activities in mouse liver

Assay	Control	CVT-E002
7-Ethoxyresorufin O-deethylation nmol/min/mg protein	0.49 ± 0.08	0.45 ± 0.08
7-Methoxyresorufin O-demethylation nmol/min/mg protein	1.49 ± 0.13	1.76 ± 0.27
Benzo(a)pyrene hydroxylation nmol/min/mg protein	1.38 ± 0.12	1.74 ± 0.17
7-Ethoxycoumarin O-deethylation nmol/min/mg protein	2.08 ± 0.39	3.53 ± 0.68
Benzphetamine N-demethylation nmol/min/mg protein	5.26 ± 0.23	6.81 ± 0.59
N-Nitrosodimethylamine N-demethylation nmol/min/mg protein	2.01 ± 0.13	2.22 ± 0.26
Erythromycin N-demethylation nmol/min/mg protein	3.82 ± 0.67	4.98 ± 0.68
Nifedipine oxidation nmol/min/mg protein	0.65 ± 0.03	0.72 ± 0.04

Mice were treated with 5 g/kg/day CVT-E002 in water by gastrogavage for three days. Results represent means ± SE of six and five mice in control and CVT-E002-treated groups, respectively.

Table 3. Effects of CVT-E002 on conjugation activities in mouse liver

Conjugation activity	Control	CVT-E002
UDP-Glucuronosyl transferase nmol/min/mg protein	42.1 ± 4.6	49.7 ± 8.2
Glutathione S-transferase µmol/min/mg protein	7.13 ± 0.58	5.91 ± 0.54

Mice were treated with 5 g/kg/day CVT-E002 suspended in water by gastrogavage for three days. Results represent means ± SE of six and five mice in control and CVT-E002-treated groups, respectively.

Many edible plants and herbs have been reported to initiate drug interactions with pharmaceutical drugs²⁸. One glass of grapefruit juice increases the serum concentration of felodipine. Guo *et al*²⁹ reported that a mixture of five main furanocoumarins isolated from grapefruit juice inhibited human liver microsomal testosterone 6β-hydroxylation activity with extent similar to that of grapefruit juice. However, the absence of any one of these furanocoumarins decreased the inhibition potency of the mixture. This report demonstrated that the effects of individual components might be different from the mixture of constituents or plant extract. St. John's Wort has been widely used as a natural antidepressant. However, the bioavailabilities of digoxin, theophylline, and cyclosporin are decreased by the concomitant use of these drugs and St. John's Wort. In the primary culture of human hepatocytes, St. John's Wort and its component hyperforin caused induction of CYP3A4³⁰ which might result in the decrease of serum concentrations of CYP3A4 substrates such as cyclosporin. These studies underscore the importance of the investigation of plant preparation and herbal extracts that human may ingest.

Our results showed that CVT-E002 had no effect on C57BL/6J mouse hepatic CYP, UGT, and GST activities, suggesting a low potential of incidence for CVT-E002-mediated metabolism-based drug interaction. Compared to the present mouse study, the treatment dosage of human is relatively low. Although it is difficult to make direct extrapolation from mouse to human, the present results nevertheless suggest a low potential of CYP-, UGT-, or GST-mediated drug interactions by CVT-E002.

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西洋參萃取物 CVT-E002 對 C57BL/6J 鼯鼠肝藥物代謝之影響

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CVT-E002 是一具專利之西洋參 (*Panax quinquefolium*) 萃取物，具有調節免疫功能之作用。細胞色素 P450 (CYP)，UDP-葡萄糖醛酸轉移 (UGT)，及 glutathione 硫-轉移 為重要之藥物代謝，藥物代謝 之調控影響是產生藥物交互作用之重要因素。為評估 CVT-E002 因影響藥物代謝 而產生藥物交互作用之可能性，因而研究 CVT-E002 對鼯鼠 C57BL/6J 肝 CYP，UGT 及 GST 之影響，結果顯示以 5 克/公斤/天 CVT-E002 連續處理鼠 3 天後，對鼠肝微粒體 CYP 及細胞色素 b_5 含量無影響，對 NADPH-CYP 還原 活性亦無影響。這處理不影響鼠肝微粒體 CYP 氧化代謝 7-ethoxyresorufin，7-methoxyresorufin，benzo(a)pyrene，7-ethoxycoumarin，benzphetamine，N-nitrosodimethylamine，erythromycin 及 nifedipine 等的活性。CVT-E002 對鼠肝微粒體 UGT 及細胞質液 GST 活性無影響。這些結果顯示 CVT-E002 在此處理劑量與時程，對鼠肝 CYP，UGT，及 GST 活性均無影響，CVT-E002 與其他藥物因影響藥物代謝，而發生交互作用之機會可能相對較低。

關鍵詞：CVT-E002，細胞色素 P450，UDP-葡萄糖醛酸轉移，glutathione 硫-轉移。