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Induction of cytochrome P450-dependent monooxygenase by extracts of the medicinal herb *Salvia miltiorrhiza*

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Abstract

The herbal medicine *Salvia miltiorrhiza* (Danshen) is currently used for the treatment of cardiovascular and cerebrovascular diseases. To assess possible herb–drug interactions, the effects of the aqueous and ethyl acetate extracts of *S. miltiorrhiza* on cytochrome P450 (CYP) were studied. Oral treatment of C57BL/6J mice with the ethyl acetate extract caused a dose-dependent increase in liver microsomal 7-methoxyresorufin *O*-demethylation (MROD) activity. The ethyl acetate extract caused an 8-, 2-, 3- and 3-fold increase in hepatic MROD, tolbutamide hydroxylation, nifedipine oxidation and warfarin 7-hydroxylation activity, respectively. However, the aqueous extract had no effects on any of the activities determined. Pharmaceutical product of *S. miltiorrhiza* extract caused a dose-dependent increase in MROD activity without affecting other activity. Immunoblot analysis of microsomal proteins showed that ethyl acetate extract-treatment elevated the protein levels of CYP1A and CYP3A. Tanshinone IIA was the main diterpene quinone in *S. miltiorrhiza*. At the dose corresponding to its content in ethyl acetate extract, tanshinone IIA-treatment increased mouse liver microsomal MROD activity. These results demonstrated that there were mouse CYP1A, CYP2C and CYP3A-inducing agents present in the ethyl acetate extract, but not in the aqueous extract, of *S. miltiorrhiza*. Tanshinone IIA played a role in the induction of CYP1A by *S. miltiorrhiza*. The CYP induction by the ethyl acetate extract and pharmaceutical product suggested that possible drug interactions between *S. miltiorrhiza* and CYP substrates should be noticed.

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Introduction

Microsomal cytochrome P450 (CYP, P450)-dependent monooxygenase is the primary enzyme system catalysing the oxidation of many endogenous and exogenous substrates, including drugs, steroids, environmental pollutants and chemical carcinogens (Guengerich 1995). Microsomal monooxygenase-catalysed oxidation requires electron transfer from NADPH to P450 by a flavoprotein NADPH-P450 reductase, and cytochrome b_5 is essential for the second electron transfer to a number of P450 forms, such as CYP2E1 and CYP3A4. P450 is responsive to the modulatory effects of natural products, including edible plants and medicinal herbs. The modulation of P450 is a main factor of herb–drug interactions (Guengerich 1997). The P450 family comprises a group of enzymes with a broad substrate specificity. The substrate specificity provides a good criterion for the study of xenobiotic influences on individual P450 forms. CYP1A2, CYP2C and CYP3A are the main P450 forms in human and rodent liver. CYP1A2, CYP2C and CYP3A constitute about 13%, 20% and 30% of the total human hepatic P450 content, respectively (Shimada et al 1994). 7-Methoxyresorufin, tolbutamide and nifedipine are the model substrates of hepatic CYP1A2, CYP2C and CYP3A, respectively (Guengerich 1995).

Salvia miltiorrhiza (Danshen) is a herbal medicine used for the treatment of coronary heart disease and cerebrovascular disease in Asia. The administration of *S. miltiorrhiza* increased the international normalized ratio (INR) and the risk of bleeding while patients were co-treated with the anticoagulant warfarin (Chan 2001). Chan

et al (1995) reported that treatment of rats with the aqueous extract of *S. miltiorrhiza* elevated the area under the plasma concentration and time curve (AUC) of warfarin, suggesting an inhibitory effect of *S. miltiorrhiza* on P450, which catalyses the oxidative metabolism of warfarin. In clinics, the racemic mixture of warfarin is used and *S*-warfarin has pharmacological efficacy higher than that of *R*-warfarin (Rettie et al 1999). In man, *S*-warfarin is mainly metabolized by CYP2C9 to form 7-hydroxywarfarin. *R*-Warfarin is metabolized by CYP1A2, CYP2C19 and CYP3A4 to form various hydroxylation products, including 6-, 7- and 8-hydroxywarfarin (Rettie et al 1989). In the roots of *S. miltiorrhiza*, tanshinone IIA is the main active diterpene quinone and constitutes up to 0.1% of the dry weight (Wu et al 1991). Tanshinone IIA has a planar structural feature of phenanthrene *o*-quinone. Our previous report showed that tanshinone IIA is a hepatic CYP1A2 inducer in C57BL/6J mice (Ueng et al 2004). However, the inductive effects of *S. miltiorrhiza* extracts were not clear. Thus, in this report, aqueous and ethyl acetate extracts were prepared to obtain the hydrophilic and hydrophobic fractions of *S. miltiorrhiza*, respectively. The effects of *S. miltiorrhiza* extracts and pharmaceutical product on monooxygenase components and the oxidation of 7-methoxyresorufin, tolbutamide, nifedipine and warfarin were investigated in C57BL/6J mice. The content of tanshinone IIA in the extracts was determined to define its role in P450 induction.

Materials and Methods

Chemicals and antibodies

Tanshinone IIA was isolated and purified from the roots of *S. miltiorrhiza* (Ryu et al 1997). The purity of tanshinone IIA was 99% as determined by HPLC and ^{1}H NMR analyses. Cytochrome *c*, 7-methoxyresorufin, NADH, NADPH, nifedipine, tolbutamide and 4-hydroxytolbutamide were purchased from Sigma-Aldrich (St Louis, MO, USA). 7-Hydroxywarfarin was purchased from Ultrafine Ltd (Manchester, UK). Monoclonal antibodies against rat CYP1A1 (MAb 1-7-1) and CYP3A (MAb 2-13-1) were generously provided by Dr Sang Shin Park (Seoul, Korea). Polyclonal peptide antibody to rat CYP2C11 was purchased from Affinity BioReagents Inc. (Golden, CO, USA).

Extract preparation

The dried roots of *S. miltiorrhiza* were purchased from a local Chinese herb store in Taipei. Aqueous extract was prepared by immersing *S. miltiorrhiza* (500 g) in 1.5 L deionized water and then the mixture was shaken in an orbital shaker at room temperature overnight. An additional 2 L de-ionized water was added and the mixture was boiled for 3 h. After filtration, the filtrate was concentrated and lyophilized to obtain the aqueous extract. Ethyl acetate extract was prepared by extracting *S. miltiorrhiza* roots with ethyl acetate at 50°C three times. This extract was concentrated under reduced pressure and then dried under vacuum. Extracts were stored at −20°C. The pharmaceutical product of *S. miltiorrhiza* extract was purchased from Sun Ten Pharmaceutical Co. (Taipei, Taiwan). There are 0.8 g *S. miltiorrhiza* concentrated decoction and 0.4 g *S. miltiorrhiza* powder in 1.2 g pharmaceutical extract.

Animal treatment and microsomal preparation

Male C57BL/6J mice, 5 weeks old, 13–16 g, were purchased from the National Laboratory Animal Center (Taipei, Taiwan, R.O.C.). The animal experiments, including handling, drug treatment and sacrifice method, were reviewed and approved by the institutional Animal Care and Use Committee of National Research Institute of Chinese Medicine. Mice were given free access to water and laboratory rodent chow (No. 5P14; PMI Feeds Inc., Richmond, IN, USA). Before experiment, mice were allowed a one-week acclimatization in the animal centre with air conditioning (25 ± 2°C) and an automatically controlled photoperiod of 12 h light daily. Aqueous extract was dissolved in water and the treatment regimen was performed according to the method of Chan et al (1995). Mice were treated with 5 g kg^{-1} aqueous extract at 0930 h and 1600 h daily for three days. The ethyl acetate extract and pharmaceutical product were suspended in corn oil and water, respectively. Mice were treated with ethyl acetate extract (0.5–3 g kg^{-1} daily) and pharmaceutical product (3 and 5 g kg^{-1} daily) for five days. The control group received the same amount of water or corn oil. Water, corn oil and the extracts were administered to mice by gastrogavage. Each group contained at least four mice. Twenty hours after the last treatment, washed microsomes were prepared from individual mouse livers by differential centrifugation at 4°C and stored at −75°C (Alvares & Mannering 1970). Enzyme assays were performed within two weeks.

Enzyme assays

P450 and cytochrome b_5 contents were determined by the spectrophotometric method of Omura & Sato (1964). NADPH-P450 reductase activity was determined following the method of Phillips & Langdon (1962) using cytochrome *c* as a substrate. 7-Methoxyresorufin *O*-demethylation (MROD) was determined by measuring the fluorescence of resorufin (Pohl & Fouts 1980). Tolbutamide hydroxylation and nifedipine oxidation were determined by HPLC analysis following the methods of Yamazaki et al (1998) and Guengerich et al (1986), respectively. Warfarin 7-hydroxylation was determined by HPLC analysis following the method of Yamazaki & Shimada (1997). Microsomal protein concentration was determined using bovine serum albumin as a standard following the method of Lowry et al (1951).

Immunoblot analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the discontinuous system of Laemmli (1970). Microsomal proteins were

electrophoresed on a 10% (w/v) polyacrylamide gel. Electrophoresis was carried out at 4°C and at 15 mA/gel during stacking and 30 mA/gel during separation. Following electrophoresis, microsomal proteins were transferred from the slab gel to a nitrocellulose membrane using the method of Towbin et al (1979). Immunodetection of P450 in liver microsomes was performed using MAb 1-7-1, MAb 2-13-1 or anti-CYP2C11 sera (Gelboin 1993; Alkayed et al 1996). Immunoreacted proteins were detected using rabbit anti-mouse IgG or goat anti-rabbit IgG conjugated with horseradish peroxidase and immunostained with a chemiluminescence detection kit from NEN Life Science Products, Inc. (Boston, MA, USA). The protein band density was analysed by densitometry using ImageMaster (Pharmacia Biotech Ltd, Uppsala, Sweden).

Quantitation of tanshinone IIA in the extracts

Aqueous and ethyl acetate extracts of *S. miltiorrhiza* were dissolved in water and methanol, respectively. Pharmaceutical product of *S. miltiorrhiza* extract (0.5 g) was extracted with 5 mL ethyl acetate twice and the filtrates were combined and dried under nitrogen gas. The dried filtrate was dissolved in methanol. Before injection onto HPLC, all samples were filtered through a 0.2 μm microspin PVDF filter. Tanshinone IIA content was determined by an HPLC system (HP1100; Agilent Technologies, Inc., DE, USA) equipped with a G1379A degasser, a G1311A QuatPump, a G1315B UV-detector and a G1329A autosampler. The constituents of the extracts were separated by a C18 column (5 μm, 4.6 × 250 mm) using a mobile phase containing 70% methanol and 30% water at 1 mL min^{-1}. Tanshinone IIA was detected by measuring the absorbance at 254 nm and quantified using purified tanshinone IIA as a standard.

Statistical analysis

The differences between > 2 sets of data were analysed by one-way analysis of variance followed by Dunnett's or Tukey's tests for multiple comparisons (SigmaStat 2.0; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

Results

To examine the effect of the aqueous extract of *S. miltiorrhiza* on warfarin hydroxylation, the treatment regimen of aqueous extract (twice daily for three days) was the same as that used by Chan et al (1995), which showed the pharmacokinetic interference of warfarin by the aqueous extract. Mice were treated with ethyl acetate extract once daily for five days following the method of Ueng et al (2004), which showed CYP1A induction by a *S. miltiorrhiza* constituent, tanshinone IIA. The aqueous extract of *S. miltiorrhiza* had no effect on mouse liver microsomal P450 or cytochrome b_5 content or NADPH-P450 reductase activity (Table 1). Aqueous extract treatment had no effect on hepatic MROD, tolbutamide hydroxylation, nifedipine oxidation or warfarin 7-hydroxylation activity. In contrast, ethyl acetate extract treatment at 3 g kg^{-1} daily for 5 days significantly increased liver microsomal P450 content (Figure 1A). Treatment with 0.5, 1 and 3 g kg^{-1} ethyl acetate extract daily for five days caused 4-, 5- and 8-fold increases in MROD activity in mouse liver, respectively (Figure 1B). The increase in

Table 1 Effect of *S. miltiorrhiza* aqueous and ethyl acetate extracts on cytochrome P450-dependent monooxygenases in mouse liver

Assays	Control	*S. miltiorrhiza*	
		Aqueous extract	Ethyl acetate extract
Cytochrome P450 (nmol (mg protein)$^{-1}$)	0.54 ± 0.02	0.51 ± 0.07	1.05 ± 0.07*
Cytochrome b_5 (nmol (mg protein)$^{-1}$)	0.36 ± 0.01	0.37 ± 0.05	0.68 ± 0.05*
NADPH-cytochrome P450 reductase (nmol min^{-1}(mg protein)$^{-1}$)	286 ± 21	311 ± 16	350 ± 16
7-Methoxyresorufin *O*-demethylation (nmol min^{-1} (mg protein)$^{-1}$)	0.71 ± 0.08	0.67 ± 0.06	5.41 ± 0.49*
Tolbutamide hydroxylation (nmol min^{-1} (mg protein)$^{-1}$)	0.48 ± 0.01	0.50 ± 0.01	0.80 ± 0.03*
Nifedipine oxidation (nmol min^{-1} (mg protein)$^{-1}$)	0.66 ± 0.09	0.68 ± 0.01	1.84 ± 0.08*
Warfarin 7-hydroxylation (pmol min^{-1} (mg protein)$^{-1}$)	1.83 ± 0.07	1.88 ± 0.06	6.03 ± 0.28*

Mice were treated with 5 g kg^{-1} twice daily aqueous extract for three days or 3 g kg^{-1} daily ethyl acetate extract for five days. Results of the treated groups represent means ± s.e.m. of 5 or 6 mice. There was no significant difference between water- and corn-oil-treated groups. Control values were obtained from the pooled data of water- and corn-oil-treated groups (n = 6). *$P < 0.05$ compared with the control value.

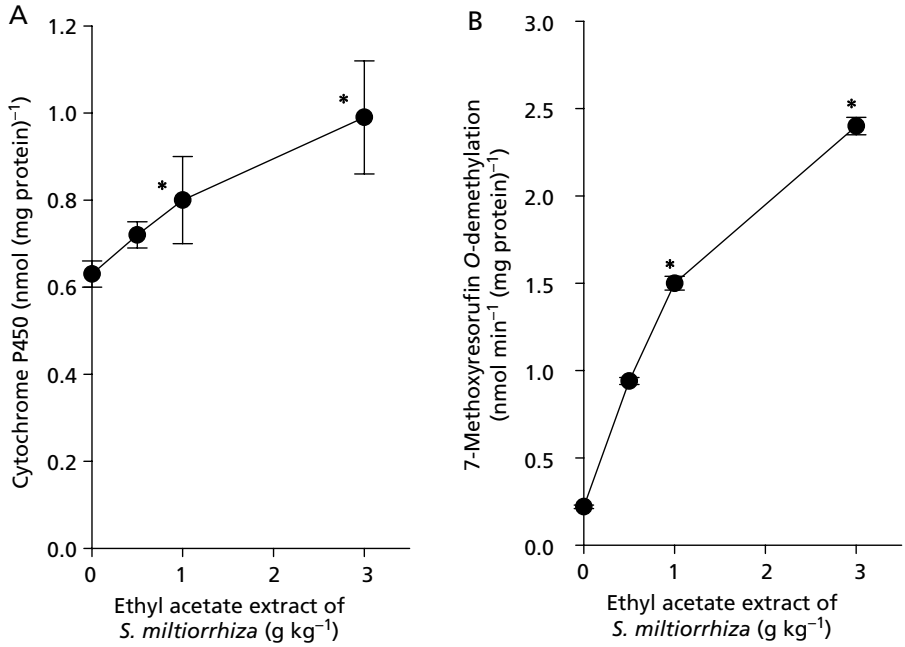

Figure 1 Dose–response of effects of the ethyl acetate extract of *S. miltiorrhiza* on liver microsomal cytochrome P450 content (A) and 7-methoxyresorufin *O*-demethylation activity (B) in mice. Mice were treated with ethyl acetate extract for five days. Results represent means ± s.e.m. of four mice. $^*P < 0.05$ compared with the control values.

MROD activity was dose-dependent. Maximal induction occurred at a dose of 3 g kg^{-1} daily. At this dose, ethyl acetate extract treatment resulted in a 35% increase in the liver-to-body-weight ratio (result not shown). Ethyl acetate extract caused a 94% and 89% increase in P450 and cytochrome b_5 content, respectively (Table 1). NADPH-P450 reductase activity was not changed. The ethyl acetate extract caused an 8-, 2-, 3- and 3-fold increase in hepatic MROD, tolbutamide hydroxylation, nifedipine oxidation and warfarin 7-hydroxylation activity, respectively. To elucidate the effect of a pharmaceutical product of *S. miltiorrhiza* extract, mice were treated with various doses of the extract for five days. Mice treated with the pharmaceutical extract of *S. miltiorrhiza* at 0 (n = 7), 3 (n = 5) and 5 g kg^{-1} daily (n = 4) had an MROD activity of 0.89 ± 0.05, 1.30 ± 0.10 and 1.42 ± 0.06 nmol min^{-1} (mg protein)$^{-1}$, respectively. The pharmaceutical extract at 3 and 5 g kg^{-1} daily caused a 47% and 60% increase in MROD activity, respectively. However, P450 and cytochrome b_5 content and tolbutamide hydroxylation, nifedipine oxidation and warfarin 7-hydroxylation activity were not affected by the treatment at either of the doses (results not shown).

Immunoblot analyses of liver microsomal proteins from control and *S. miltiorrhiza*-treated mice showed that aqueous extract treatment had no effect on the levels of CYP1A- and CYP3A-immunoreactive proteins (Figure 2). The ethyl acetate extract caused a 2-fold increase in the band intensity of CYP1A2 (Figure 2, upper panel). The ethyl acetate extract also stimulated the expression of CYP1A1. CYP1A1 had a mobility slower than that of CYP1A2 and was undetectable in control mouse liver microsomes under the experimental conditions reported previously (Ueng et al

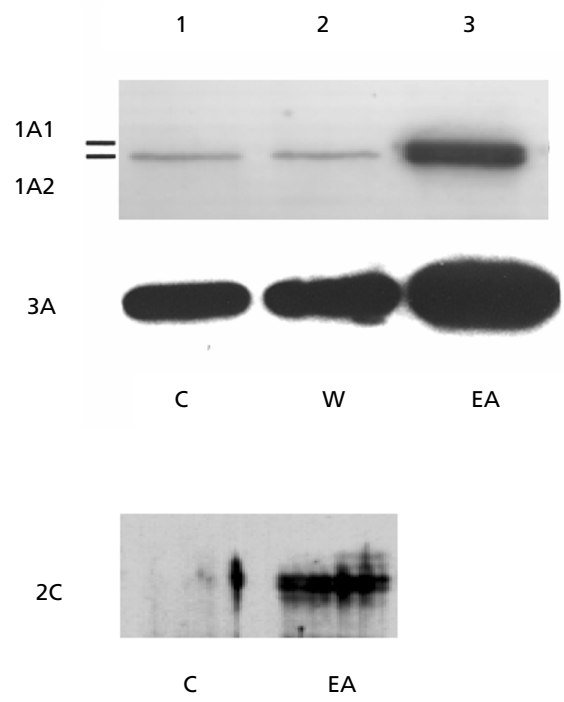

Figure 2 Immunoblot analyses of liver microsomal cytochrome P450 protein levels from control (C), *S. miltiorrhiza* aqueous extract (W)- and *S. miltiorrhiza* ethyl acetate extract (EA)-treated mice. Liver microsomal proteins (50 μg) were separated on a 10% polyacrylamide gel and transferred to nitrocellulose membrane. The CYP1A- and CYP3A-immunoreactive proteins were detected by their respective monoclonal antibodies, MAb1-7-1 and MAb2-13-1. The CYP2C-immunoreactive protein was detected by polyclonal anti-CYP2C11 sera. The immunoreactive bands were visualized by a chemiluminescence detection kit.

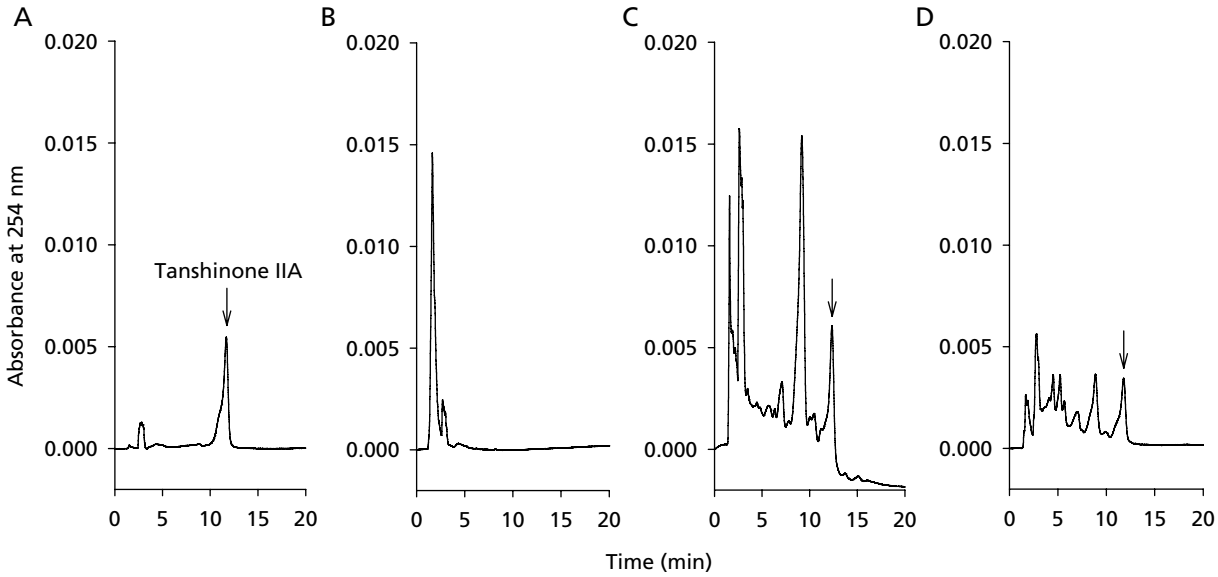

Figure 3 HPLC chromatograms of tanshinone IIA present in extracts and the pharmaceutical extract of *S. miltiorrhiza*. A. Chromatogram of tanshinone IIA. B, C and D. Chromatograms of aqueous extract (0.03 mg), ethyl acetate extract (0.03 mg) and the pharmaceutical product (0.01 g), respectively. The fraction appearing at 12 min, as indicated by an arrow, was tanshinone IIA.

1999). Ethyl acetate extract treatment caused a 66% increase in the level of a CYP3A-immunoreactive protein (Figure 2, middle panel). The level of CYP2C-immunoreactive protein was increased by the ethyl acetate extract to a detectable level (Figure 2, bottom panel).

HPLC analysis was conducted to determine the content of tanshinone IIA in the extracts (Figure 3). Tanshinone IIA was not detectable in the aqueous extract. One gram of the ethyl acetate extract and pharmaceutical product contained 14.6 mg and 0.03 mg tanshinone IIA, respectively. To elucidate the role of tanshinone IIA in the induction of CYP1A by *S. miltiorrhiza* extracts, treatments with 40 and 0.15 mg kg^{-1} tanshinone IIA were equivalent to treatment with 3 g kg^{-1} ethyl acetate extract and 5 g kg^{-1} pharmaceutical extract in terms of the amount in tanshinone IIA of *S. miltiorrhiza* administered to mice, respectively. Treatment with tanshinone IIA at these doses for five days had no effect on microsomal protein and total P450 contents in mouse liver (Table 2). Treatment with 0.15 mg kg^{-1} tanshinone IIA had no effect on hepatic MROD activity. However, 40 mg kg^{-1} tanshinone IIA caused a 66% increase in hepatic MROD activity.

Table 2 Effect of tanshinone IIA on cytochrome P450-dependent monooxygenases in mouse liver

	Control	Tanshinone IIA	
		0.15 mg kg^{-1}	40 mg kg^{-1}
Cytochrome P450 (nmol (mg protein)$^{-1}$)	0.48 ± 0.10	0.39 ± 0.04	0.49 ± 0.03
7-Methoxyresorufin *O*-demethylation (nmol min^{-1} (mg protein)$^{-1}$)	0.50 ± 0.10	0.46 ± 0.08	0.83 ± 0.08*

Mice were treated with various daily doses of tanshinone IIA for five days. Results represent means ± s.e.m. of four mice. *$P < 0.05$ compared with the control values.

Discussion

S. miltiorrhiza is reported to have beneficial effects on reduction of blood cholesterol, which is a risk factor for cardiovascular and cerebrovascular diseases. The hot-water extract reduced serum cholesterol concentration and inhibited lipid peroxidation in rats with liver fibrosis induced by bile-duct ligation and scission (Nan et al 2001). The extract prepared by water–ethanol (4:1, v/v) extraction decreased plasma cholesterol concentration and reduced lipid peroxidation in rabbits fed a high-cholesterol diet (Wu et al 1998). Our results revealed that the ethyl acetate extract caused at least a 2-fold increase in the oxidative activity toward the respective model substrates of CYP1A, CYP2C and CYP3A (Table 1) in mouse liver. The pharmaceutical extract also stimulated CYP1A catalytic activity. In human liver, CYP3A4, but not CYP1A2 or CYP2C9, catalyses the 6α- and 4β-hydroxylation of cholesterol and bile acid 6α-hydroxylation (Wietholtz et al 1996; Bodin et al 2001). The induction of CYP3A by the ethyl acetate extract suggested that *S. miltiorrhiza* might increase cholesterol metabolism and then decrease blood cholesterol concentration. However, the effects of *S. miltiorrhiza* on other cholesterol-metabolizing enzymes, such as human CYP7A and CYP27, are not reported. Human and mouse CYP1A2 and CYP2C are involved in the

metabolism of arachidonic acid to form epoxidation and hydroxylation metabolites, such as 11- and 12-epoxyeicosatrienoic acid, which affect vascular tone and ion transport (Rifkind et al 1995; Wang et al 2004). It is of interest to characterize the effect of *S. miltiorrhiza* on the metabolic profile of arachidonic acid in the future.

To assess the possible herb–drug interaction, mice were treated with the aqueous extract twice a day for three days and with the ethyl acetate extract once a day for five days. Although different treatment regimens may cause differential results, our results demonstrated that inducing agents of CYP1A, CYP2C and CYP3A were mainly present in the hydrophobic ethyl acetate extract, but not in the hydrophilic aqueous extract of *S. miltiorrhiza*. Drugs such as caffeine and paracetamol (acetaminophen) are CYP1A2 substrates (Guengerich 1995). The anticoagulant warfarin is mainly metabolized by CYP2C9. CYP3A4 catalyses the oxidation of a series of drugs, including antibiotics, anti-hypertensives and anti-inflammatory drugs (Guengerich 1995). For the treatment of cardiovascular or cerebrovascular diseases, these drugs are possibly used in patients pre- or concomitantly treated with a herbal remedy containing *S. miltiorrhiza*. Although the ethyl acetate extract is not used directly as pharmaceutical preparation, the pharmaceutical preparation protocol should be defined and possible herb–drug interactions should be noted, especially when the powder of dried roots is directly used.

Our immunoblot analyses showed that the ethyl acetate extract elevated mouse hepatic CYP1A1, CYP1A2, CYP2C and CYP3A protein levels (Figure 2). Our previous report showed that the main active diterpene quinone of *S. miltiorrhiza*, tanshinone IIA, at doses higher than $50\,mg\,kg^{-1}$ elevated hepatic CYP1A activity (Ueng et al 2004). In accordance with the content of tanshinone IIA in the ethyl acetate extract, treatment of mice with $40\,mg\,kg^{-1}$ tanshinone IIA caused a 66% increase of hepatic MROD activity (Table 2). Tanshinone IIA contributed at least in part to the induction of mouse hepatic CYP1A2 by *S. miltiorrhiza* ethyl acetate extract. However, at a dose as high as $75\,mg\,kg^{-1}$ tanshinone IIA, there was only a two-fold increase in MROD activity and this treatment had no effects on tolbutamide hydroxylation or nifedipine oxidation activity (Ueng et al 2004). Thus, there are P450-inducing constituents other than tanshinone IIA present in *S. miltiorrhiza*. HPLC anlaysis of the constituents of the extracts showed that additional peaks were present at the retention time of 5–10 min (Figures 3C and D). It will be interesting to determine their effect on P450 induction and to identify the P450-inducing constituents of *S. miltiorrhiza* in the future.

Chan et al (1995) reported that an aqueous extract of *S. miltiorrhiza* increased and decreased the AUC and clearance of warfarin in rats, respectively, suggesting inhibition of warfarin metabolism by *S. miltiorrhiza*. Qiao et al (2003) reported that the aqueous extract of *S. miltiorrhiza* stimulated rat hepatic P450 content and erythromycin *N*-demethylation activity and decreased the AUC value of diazepam. In contrast, our results showed that the aqueous extract had no effect on warfarin 7-hydroxylation and nifedipine oxidation activity (Table 1). Chan et al (1995) prepared the aqueous extract using water only, as did we. However, Qiao et al (2003) prepared the aqueous extract using both water and ethanol. The constituents of these aqueous extracts could be different due to different preparation methods. On the other hand, P450 catalytic activity and its responsiveness to xenobiotic modulation show species differences (Bogaards et al 2000; Handschin & Meyer 2003). These differences may contribute to differential P450 modulatory effects by xenobiotics. In our study, the pharmaceutical product contained *S. miltiorrhiza* concentrated decoction and *S. miltiorrhiza* powder. The decoction is basically prepared from a water extract but details of the protocol are not available. Our report is the first to demonstrate that this pharmaceutical product could elevate mouse CYP1A activity. Thus, standardized preparation of herbal remedy is an important factor to consider in the regulation of use of *S. miltiorrhiza*. The suggested daily dose of pharmaceutical product in man is $0.4 \sim 1.8\,g$. This dose is about $1 \sim 6\%$ of that used in mice showing MROD induction. The extrapolation from experimental animal to man needs further study.

Conclusion

Our study demonstrated that potent mouse CYP1A, CYP2C and CYP3A inducer(s) were present in the ethyl acetate extract of the roots of *S. miltiorrhiza*. In contrast, the aqueous extract had no effect on the catalytic activity of these P450s. The pharmaceutical product of *S. miltiorrhiza* extract also increased CYP1A activity.

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