**Regular Article**

**Induction of Hepatic Cytochrome P450s by the Herbal Medicine *Sophora flavescens* Extract in Rats: Impact on the Elimination of Theophylline**

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**Summary:** The roots of *Sophora flavescens* (Sf) have been widely used as a herbal medicine for the treatment of diarrhea, gastrointestinal hemorrhage, and eczema. Cytochrome P450 (P450) forms including CYP1A2, CYP2B, CYP2E1, and CYP3A participate in the oxidative metabolism of theophylline, which is an important bronchodilation agent with a narrow therapeutic index. To assess the interaction of Sf with theophylline, the effects of Sf extract on theophylline-metabolizing P450s and on the pharmacokinetic profile of theophylline were investigated in male Sprague-Dawley rats. Oral treatment of rats with the Sf extract caused dose-dependent increases of liver microsomal oxidation activities toward 7-ethoxyresorufin, 7-pentoxyresorufin, and nifedipine. However, nitrosodimethylamine N-demethylation activity was not affected. The ingestion of Sf extract stimulated theophylline 8-oxidation and N-demethylation activity was not affected. The increases of oxidative activities were in consensus with the elevation of the protein levels of CYP1A2, CYP2B1/2, CYP2C11, and CYP3A. Sf-treatment increased the clearance of theophylline and decreased the area under the concentration-time curve (AUC) and the area under the moment curve (AUMC). These results demonstrate that Sf reduces blood theophylline concentration through facilitating the elimination of theophylline. In patients taking Sf, possible P450 induction-induced drug interaction should be noted to decrease the risk of therapeutic failure or adverse effects resulting from the use of additional therapeutic agents.

**Keywords:** *Sophora flavescens*; theophylline; liver; cytochrome P450; pharmacokinetic interaction

**Introduction**

Sophorae radix (kushen), the dry roots of *Sophora flavescens* (Sf), has been widely used as a Chinese herbal medicine in Asia for the treatment of diarrhea, gastrointestinal hemorrhage, and eczema. In rats, ethanol-induced gastric ulcer could be diminished by the methanol extract of Sf. Recently, Sf has been reported to show anti-inflammatory effects through inhibition of the NF-κB/IκB signal pathway, and it had a beneficial effect in improving respiratory function in three years of follow-up in 14 asthma patients. Our previous report demonstrated that administration of the pharmaceutical product of Sf extract to mice at a daily dose of either 3 g/kg for 3 days or 0.18 g/kg (human equivalent dose) for 10 days increased the activity and expression of Cyp1a2,5) which is primary cytochrome P450 (P450) form that catalyzes the oxidative metabolism of the bronchodilator theophylline. Matrine and oxymatrine are the main quinolizidine alkaloid constituents of Sf and showed a variety of pharmacological activities including anti-inflammatory, antipyretic and hepatoprotective effects. Oxymatrine contributes at least in part to the induction of mouse Cyp1a2 by Sf extract.5) The active tea alkaloid theophylline is widely used for the treatment of acute asthma and chronic obstructive
pulmonary disease.\textsuperscript{9} However, theophylline has a narrow therapeutic index; it is currently used as a third-line agent due to the frequency of side effects and its relative low efficacy. Careful monitoring of plasma theophylline concentration may be essential, especially in elderly patients treated with multiple drugs. The bioavailability of theophylline through oral administration is close to unity in rats and humans,\textsuperscript{9} and more than 90\% of theophylline is metabolized in the liver. P450 occupies the rate limiting step in the phase I oxidative metabolism of theophylline.\textsuperscript{10,11} In addition to its effect on theophylline, P450 plays a primary role in the oxidation of numerous xenobiotics with a variety of structures, including drugs, pollutants, and natural products.\textsuperscript{12} The oxidations catalyzed by the microsomal P450-dependent monoxygenase system require the sequential transfer of two electrons from NADPH-P450 reductase to P450; the second electron can also be transferred by cytochrome bs.\textsuperscript{13}

In rats, theophylline is 8-oxidized by P450 enzymes CYP1A2, CYP2B1, CYP2E1, and possibly CYP3A1/2 to form 1,3-dimethyluric acid, which is the main urinary metabolite.\textsuperscript{11,14–16} In human liver, CYP1A2 and CYP2E1 are mainly involved in the oxidative metabolism of theophylline.\textsuperscript{10,17,18} Among hepatic P450 forms, CYP1A2 had high affinity for theophylline oxidation and showed the highest intrinsic clearance.\textsuperscript{17,18} CYP1A2 also participates in the N-demethylation of theophylline to produce 1- and 3-methylxanthine.\textsuperscript{19} Correlation analysis of microsomal activities suggested that CYP3A4 may also participate in theophylline 8-hydroxylation, but with relatively low affinity and a lower contribution to microsomal metabolic formation.\textsuperscript{10,19}

One of the main factors causing drug interaction with theophylline is activity changes in theophylline-metabolizing P450s by xenobiotics including cigarette smoke and medicines.\textsuperscript{5,20} The elevation of theophylline elimination diminishes the pharmacological efficacy and may cause therapeutic failure in asthma patients. Asthma patients need long-term health care and are likely to try alternative therapies, such as herbal medicines. Before or during treatment with theophylline, patients may take a herbal remedy such as Sf for the treatment of symptoms as described above. To understand the modulatory effect of Sf extract on theophylline metabolism, the effects of Sf extract on rat hepatic CYP1A, CYP2B1/2, CYP2E1, and CYP3A and theophylline pharmacokinetic parameters were investigated in male Sprague-Dawley rats.

**Materials and Methods**

**Chemicals, enzymes, and antibodies:** Cytochrome c, 7-ethoxyresorufin, glucose-6-phosphate dehydrogenase, NADH, NADPH, nileadpine, 7-pentoxyresorufin and 1,3-dimethyluric acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Monoclonal antibodies against rat CYP1A1/2 (MAB 1-7-1), CYP2E1 (MAB 1-98-1) and CYP3A (MAB 2-13-1) were generously provided by Dr. Sang Shin Park (Seoul, Korea). Goat polyclonal antibody against rat CYP2B1/2 was purchased from Daiichi Pure Chemicals, Japan (BD Biosciences Company-Gentest, NJ, U.S.A.). Rabbit polyclonal antibody against rat CYP2C11 was purchased from NOSAN (Yokohama, Japan). Rabbit polyclonal antibody against human NADPH-P450 reductase was kindly provided by Dr. Yun (Gwangju, Korea). 1-Methylxanthine and 3-methylxanthine were purchased from Fluka Chemie Sigma-Aldrich (Buchs, Switzerland).

**S. flavescens extract:** A powdered concentrate of Sf decoction was used as the Sf extract in this report. The powdered concentrate of Sf decoction (lot no. 136002) was purchased from Sun Ten Pharmaceutical (Taipei, Taiwan) with a certificate of good manufacturing practice. One gram Sf extract contained 0.68 g concentrated decoction of the roots of Sf and 0.32 g starch. The contents of alkaloids matairesin and oxytraxil were 0.34 mg and 6.6 mg in one gram Sf extract, respectively.\textsuperscript{5}

**Animal treatment and microsomal preparation:** All experimental protocols involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the National Research Institute of Chinese Medicine. Male Sprague-Dawley rats (5–7 weeks old, weighing 110–200 g) were purchased from the Laboratory Animal Center, National Yang-Ming University, Taipei. Before experimentation, rats were allowed a one-week acclimation period with air conditioning, free access to laboratory rodent chow (#5P14; PMT Feeds, Richmond, IN, U.S.A.), and an automatically controlled photoperiod of 12 hr light daily. Sf extract was suspended in water using a mortar and pestle, and was mixed by vortexing before administration. The control group received the same volume of water per kg body weight. Water or extract were administered to rats by gastrogavage for three days at the dosage as described in the figure legends and tables. Rats were euthanized by CO\textsubscript{2} asphyxiation and livers were removed 22 hours after the last administration of Sf extract. Washed microsomes were prepared from individual rat liver by differential centrifugation at 4°C and stored at −75°C.\textsuperscript{21} Enzyme activities were determined within two weeks.

**Serum biochemical parameters:** Before CO\textsubscript{2} asphyxiation, rats were treated intraperitoneally with a single injection of 80 mg/kg pentobarbital. Blood was collected by heart puncture under anesthesia and serum was obtained by centrifugation of blood at 3,000 rpm for 10 min at room temperature. Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and concentrations of blood urea nitrogen (BUN) and creatinine were determined using analysis kits designed for the Fujifilm Dri-Chem 3000 colorimetric analyzer (Fujifilm, Saitama, Japan).

**Enzyme assays:** Microsomal contents of P450 and...
cytochrome $b$, were determined by the spectrophotometric method of Omura and Sato. NADPH-P450 reductase activity was determined following the method of Phillips and Langdon using cytochrome $c$ as a substrate. 7-Ethoxyresorufin O-deethylation (EROD), 7-methoxyresorufin O-demethylation (MROD) and 7-pentoxyresorufin O-dealkylation (PROD) activities were determined by measuring the fluorescence of resorufin. Nitrosodimethyamine N-demethylation activity was determined by measuring the formaldehyde formation using Nash’s reagent. Nifedipine oxidation activity (NFO) was determined following the methods of Guengerich et al. Theophylline oxidation and N-demethylation activities were determined following the method of Rasmussen et al. Microsomal protein concentration was determined using bovine serum albumin as a standard following the method of Lowry et al.

**Immunoblot analyses:** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the discontinuous system of Laemmli. Microsomal proteins were electrophoresed on a 10% (w/v) polyacrylamide gel. Electrophoresis was carried out at $8^\circ\text{C}$ and at 15 mamp/gel during stacking and 30 mamp/gel during separation. Following electrophoresis, microsomal proteins were transferred from the slab gel to a nitrocellulose membrane using the method of Towbin et al. Immunodetection of P450 in liver microsomes was performed using monoclonal antibodies against rat CYP1A1/2 (MAb 1–7–1), CYP2E1 (MAb 1–98–1), and CYP3A (MAb 2–13–1) and polyclonal antibodies against rat CYP2B1/2 and CYP2C11. Immunoreacted proteins were detected using horseradish peroxidase-conjugated rabbit anti-mouse or anti-goat IgG and then immunostained with a chemiluminescence detection kit purchased from PerkinElmer LAS (MA, U.S.A.). The protein band density was analyzed by densitometry using ImageMaster (Pharmacia Biotech, Uppsala, Sweden).

**Pharmacokinetic analysis:** Six hours after the last treatment with Sf extract, access to the diet was removed and only water was provided. Eighteen hours later, rats were anesthetized with pentobarbital and then the femoral vein was cannulated with a PE-SP45 tube. Rats were treated with theophylline at 2 mg/kg intravenously via the femoral vein. Blood samples (about 0.3 ml) were collected from the heart as indicated in the results. Serum theophylline concentration was determined by the HPLC method as modified by Han and Lee. Caffeine was used as an internal standard. After protein denaturation using an equal volume of acetonitrile, the supernatant was dried under nitrogen gas, redissolved in the mobile phase, and subjected to HPLC (HP1100, Agilent Technologies, DE, USA) analysis using a C18 column (Cosmosil, 5C18-AR-II, Waters, Nacalai Tesque, Kyoto, Japan). The mobile phase consisted of 5% acetonitrile in 10 mM sodium acetate (pH 5.0) at a flow rate of 1 mL/min and theophylline was detected by measuring the absorbance at 280 nm. The coefficients of variations of the assay (intra- and inter-day) were less than 5% (standard deviation/mean) × 100%. The pharmacokinetic parameters were analyzed using WinNonLin Standard (version 1.1, Pharsight, CA, U.S.A.) according to the two-compartment open model. The fitting results generated parameters including the area under drug concentration vs time (AUC), area under the moment curve (AUMC), the distribution rate constant from the central compartment to the peripheral compartment (K12) and from the peripheral compartment to the central compartment (K21), the elimination rate constant (K10), the elimination half-life from the central compartment ($T_{1/2}$), the half-life of the terminal elimination phase ($T_{1/2}$), the distribution phase ($T_{1/2}$), distribution volume at steady state (Vss), clearance (CL), and mean residence time (MRT).

**Statistical analysis:** The statistical analysis was performed with SPSS version 10.0 (SPSS, Chicago, IL, USA). The differences between >2 sets of data (control and groups treated with various doses of Sf extract) were analyzed by one-way ANOVA followed by Dunnnett’s test for multiple comparisons. The statistical significance of differences between controls and treated groups was evaluated by Student’s t-test. p < 0.05 was considered as statistically significant.

**Results**

**Dose response of effects of Sf extract on P450 activities:** The same lot of Sf extract was used for all experiments in the current study. To analyze the dose dependence of the effects of Sf extract on hepatic P450 activities, rats were treated with 0.25–3 g/kg Sf extract once daily for 3 days. The ingestion of Sf extract did not significantly alter rat body and liver weights (data not shown). Sf extract at 0.25 g/kg did not affect EROD, PROD or NFO activities. Sf extract at 0.5, 1 and 3 g/kg increased hepatic EROD activity by 16%, 100% and 132%, respectively (Fig. 1). Treatment with 1 and 3 g/kg/day Sf extract caused 55% and 90% increases in PROD activity, 122% and 93% increases in NFO activity, and 82% and 69% increases in the formation rates of the sum of theophylline 8-oxidation and N-demethylation metabolites, respectively. These increases in activity showed dose dependence. Thus, in the following studies, rats are treated with 3 g/kg Sf extract, which causes the maximal induction of P450 activities.

**Effects of Sf on P450-dependent monoxygenase components and activities:** Administration of 3 g/kg/day Sf extract to rats by gastrogavage for 3 days did not cause significant changes in the activities of serum hepatotoxicity biomarkers AST (control group: 254 ± 51 U/L; Sf-treated group: 214 ± 41 U/L) and ALT (control
Fig. 1. Dose-response curves of the effect of *S. flavescens* extract on hepatic 7-ethoxyresorufin O-deethylation, pentoxyresorufin O-dealkylation, nifedipine oxidation and theophylline oxidation activities. Rats were treated with increasing doses of *S. flavescens* extract as indicated for 3 days. Results represent mean ± SE of 3 rats. *Asterisks represent values significantly different from the control values, p < 0.05.

**Table 1. Effects of *S. flavescens* extract on cytochrome P450-dependent monooxygenase components in rat liver**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Control</th>
<th><em>S. flavescens</em> extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomal protein (mg/g liver)</td>
<td>20.7 ± 1.1</td>
<td>23.9 ± 1.2</td>
</tr>
<tr>
<td>Cytochrome P450 (nmol/mg protein)</td>
<td>0.64 ± 0.03</td>
<td>0.86 ± 0.06*</td>
</tr>
<tr>
<td>Cytochrome b5 (nmol/mg protein)</td>
<td>0.30 ± 0.02</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>NADPH-Cytochrome c reductase (nmol/min/mg protein)</td>
<td>145 ± 17</td>
<td>167 ± 8</td>
</tr>
</tbody>
</table>

Data represent the mean ± SEM of 3 rats. Rats were treated with 3 g/kg/day *S. flavescens* extract for 3 days. *Asterisks represent values significantly different from the control values, p < 0.05.

The interaction of *Sophora flavescens* with Theophylline group: 43 ± 7 U/L; Sf-treated group: 37 ± 6 U/L) nor in renal toxicity markers, i.e., serum concentrations of BUN (control group: 15.7 ± 1.8 mg/dL; Sf-treated group: 14.3 ± 0.8 mg/dL) and creatinine (control group: 0.6 ± 0.1 mg/dL; Sf-treated group: 0.6 ± 0.0 mg/dL). To elucidate the effects of Sf on monooxygenase components, microsomal contents of P450 and cytochrome b5 as well as the cytochrome c reduction activity of NADPH-P450 reductase were determined. Treatment with 3 g/kg/day Sf extract significantly increased liver microsomal P450 content by 34%, whereas cytochrome b5 content and NADPH-cytochrome c reductase activity were not affected (Table 1). Treatment of rats with 3 g/kg Sf extract

**Table 2. Effects of *S. flavescens* extract on cytochrome P450-dependent monooxygenase activities in rat liver**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control</th>
<th><em>S. flavescens</em> extract</th>
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</thead>
<tbody>
<tr>
<td>7-Ethoxyresorufin O-deethylation</td>
<td>0.40 ± 0.04</td>
<td>0.74 ± 0.04*</td>
</tr>
<tr>
<td>7-Methoxyresorufin O-demethylation</td>
<td>0.36 ± 0.01</td>
<td>0.49 ± 0.03*</td>
</tr>
<tr>
<td>7-Pentoxyresorufin O-dealkylation</td>
<td>0.12 ± 0.02</td>
<td>0.24 ± 0.01*</td>
</tr>
<tr>
<td>Nitrosodimethylamine</td>
<td>0.807 ± 0.044</td>
<td>0.962 ± 0.046</td>
</tr>
<tr>
<td>N-demethylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theophylline oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylxanthine (pmol/min/mg protein)</td>
<td>12.9 ± 1.6</td>
<td>39.6 ± 3.6*</td>
</tr>
<tr>
<td>1-Methylxanthine (pmol/min/mg protein)</td>
<td>25.9 ± 2.4</td>
<td>37.2 ± 2.0*</td>
</tr>
<tr>
<td>1,3-Dimethyluric acid (pmol/min/mg protein)</td>
<td>105.2 ± 7.5</td>
<td>167.1 ± 11.9*</td>
</tr>
</tbody>
</table>

Data represent the mean ± SEM of 3 rats. Rats were treated with 3 g/kg/day *S. flavescens* extract for 3 days. *Asterisks represent values significantly different from the control values, p < 0.05.
resulted in 85%, 37%, 100%, and 93% increases in liver microsomal EROD, MROD, PROD and NFO activities, respectively (Table 2). However, Sf extract had no effect on nitrosodimethylamine N-demethylation activity. In the oxidation of theophylline, Sf extract elevated the formation rates of 3-methylxanthine, 1-methylxanthine, and 1,3-dimethyluric acid by 206%, 44%, and 59%, respectively. The sum of the formation of these theophylline metabolites was elevated by 69%.

**Immunoblot analyses of microsomal P450s:** To illustrate the P450 regulatory mechanism at the protein level, immunoblot analyses of liver microsomal CYP1A, CYP2B1/2, CYP2C11, CYP2E1, and CYP3A were performed. CYP1A2 was essentially the only CYP1A member expressed in the liver samples of vehicle-control rats.34) Our results of immunoblot analysis showed that Sf extract caused a 35% increase of CYP1A2 protein level (Fig. 2). Consistent with the report of Hirasawa et al.,35) CYP2B1 was hardly detected in control rat liver (Fig. 2). The band intensities of CYP2B1 and CYP2B2 were combined to calculate the induction of CYP2B1/2 in densitometric analysis. Sf extract caused a 14-fold increase of CYP2B-immunoreactive protein bands. In addition, Sf elevated the protein levels of CYP2C11 and CYP3A by 20% and 97%, respectively. Consistent with the unchanged nitrosodimethylamine N-demethylation and NADPH-cytochrome c reductase activities after Sf-treatment, the protein levels of CYP2E1 and NADPH-P450 reductase were not affected by Sf.

**Effect of Sf extract on the pharmacokinetic parameters of theophylline:** Pharmacokinetic analysis of serum theophylline concentration was performed to assess the pharmacokinetic interaction of theophylline with Sf extract. In control rats, the plot of serum theophylline concentration versus time showed a biphasic pattern with a fast distribution followed by a slower elimination phase (Fig. 3A). Our results showed that ad-

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**Table 3. The effect of S. flavescens extract on the pharmacokinetic parameters of theophylline**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>S. flavescens extract</th>
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<tbody>
<tr>
<td>AUC (µg · min/mL)</td>
<td>316 ± 7</td>
<td>244 ± 18*</td>
</tr>
<tr>
<td>AUMC (mg · min²/mL)</td>
<td>57.1 ± 2.2</td>
<td>36.4 ± 5.7*</td>
</tr>
<tr>
<td>CL (mL/kg/min)</td>
<td>6.34 ± 0.15</td>
<td>8.29 ± 0.644*</td>
</tr>
<tr>
<td>K10 (min⁻¹)</td>
<td>0.009 ± 0.000</td>
<td>0.011 ± 0.001*</td>
</tr>
<tr>
<td>K12 (min⁻¹)</td>
<td>0.071 ± 0.010</td>
<td>0.057 ± 0.029</td>
</tr>
<tr>
<td>K21 (min⁻¹)</td>
<td>0.123 ± 0.015</td>
<td>0.101 ± 0.049</td>
</tr>
<tr>
<td>(T1/2)K10 (min)</td>
<td>79.0 ± 4.6</td>
<td>65.4 ± 3.3*</td>
</tr>
<tr>
<td>(T1/2)K12 (min)</td>
<td>3.6 ± 0.3</td>
<td>6.3 ± 2.1</td>
</tr>
<tr>
<td>(T1/2)K21 (min)</td>
<td>127.6 ± 3.5</td>
<td>105.9 ± 10.1b</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>181 ± 4</td>
<td>147 ± 1.4</td>
</tr>
<tr>
<td>Vss (mL/kg)</td>
<td>1146 ± 33</td>
<td>1205 ± 54</td>
</tr>
</tbody>
</table>

Rats were pre-treated with 3 g/kg/day S. flavescens for 3 days. The control group received water only instead of S. flavescens. Pharmacokinetic parameters were determined according to the two-compartment open model as described in the Materials and Methods. Results are presented as the mean ± SEM of the fitting data of 3 rats. *p<0.05 compared to control group. b0.05<p<0.1 compared to control group.

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**Fig. 2. Immunoblot analyses of microsomal P450 enzymes in rat liver**

Microsomal proteins (25 µg in each well) were loaded on a polyacrylamide gel. Electrophoresis and immunodetection were carried out as described in the Materials and Methods section. Lanes 1 and 2 contained proteins isolated from control (C) and S. flavescens extract (Sf)-treated rats, respectively. CPR, NADPH-P450 reductase.

**Fig. 3. Panel (A) shows the biphasic pattern of the representative plot of mean unbound serum theophylline concentration-time curves in control rats**

The solid lines represent the best fit as determined by linear regression analysis. Panel (B) shows the theophylline concentration-time curves in control (○) and S. flavescens (●)-treated rats. Rats were treated with 3 g/kg/day S. flavescens extract for 3 days and then 2 mg/kg theophylline was injected via the femoral vein after anesthetization by pentobarbital on the fourth day. Control group received water only instead of S. flavescens. Data are presented as the mean±SEM of three rats. *Asterisks represent values significantly different from the control values, p<0.05.
ministration of Sf extract to rats changed the pharmacokinetic profile of theophylline (Fig. 3B). Serum concentrations of theophylline were significantly decreased after 60 min in Sf-treated rats. Sf-treatment significantly decreased AUC and AUUMC by 23% and 36%, respectively (Table 3). The clearance of theophylline was significantly increased by 31% without affecting \( \text{Vss} \), \( K_{12} \), \( K_{21} \), or \( (T_{1/2})_{b} \) (\( p > 0.3 \)). The estimated mean elimination rate constant (\( K_{10} \)) was marginally increased by 22% (\( p = 0.06 \)) but this change did not show statistical significance. The half-lives of elimination (\( T_{1/2} \)) and the terminal elimination phase (\( T_{1/2b} \)), and MRT were also marginally decreased by 17% (\( p = 0.08 \)), 17% (\( p = 0.06 \)), and 19% (\( p = 0.08 \)), respectively. However, these differences between control and Sf-treated rats did not show statistical significance.

**Discussion**

To prevent possible adverse effects in patients, herb-drug interaction has been recognized as a major concern for the safe application of herbal medicines. In humans, the suggested daily dose of the Sf extract used in our study is 0.4–1.2 g/day. According to the surface area ratio of rats to humans, the dosage coefficient for rat (0.2 kg) to human (60 kg) was 6.25. Thus, 1.2 g/day in a 60 kg patient is equivalent to 0.125 g/kg/day in rats. Our results showed that rat EROD activity was increased by 3-day treatment with Sf extract at dosages greater than 0.5 g/kg in rats. However, in humans, the regimen for Sf extract is suggested to be up to 10 days. For application in asthma patients, participants were treated with an Sf extract with high content of matrine and oxymatrine (1.8–3.2%) at an initial daily dose of 4 g dried root for 3 months and then at stepwise decreased doses for up to 27 months. A previous mouse study demonstrated that hepatic EROD activity was elevated by Sf extract under the treatment regimen of 3-day treatment at 3 g/kg or 10-day treatment at a human equivalent dose of 0.18 g/kg. Long-term and consecutive treatment with Sf at a lower dose showed inductive potential toward mouse Cyp1a similar to the effect of Sf at a higher dose for a shorter treatment period. Treatment with Sf at a high dose of 3 g/kg/day for 3 consecutive days in our previous report demonstrated that not only Cyp1a2 but also Cyp2b9/10 and Cyp3a induction were caused by Sf extract ingestion in male mice. In this rat study, liver microsomal EROD, MROD, PROD, and NFO activities were stimulated after ingestion of Sf extract at 3 g/kg/day for 3 days, whereas nitrosodimethylamine N-demethylation activity was not affected. 7-Ethoxyresorufin and 7-methoxyresorufin were preferentially oxidized by CYP1A and induced by CYP1A inducer. Both rat CYP1A2 and CYP2B1 had high PROD activity. Nitrosodimethylamine is a selective substrate for CYP2E1. Nifedipine oxidation is catalyzed almost equally by rat CYP2C11 and CYP3A1/2 and to a less extent, by CYP1A. Our results of immunoblot analyses revealed that rat CYP1A2, CYP2B1/2, CYP2C11, and CYP3A1 protein levels were elevated after oral administration of Sf extract. The increases of EROD/MROD, PROD, and NFO activities were in consensus with the elevated protein levels of CYP1A2, CYP2B1/2, CYP2C11, and CYP3A1. These results demonstrate that Sf appeared to show the ability to induce CYP1A, CYP2B, and CYP3A protein expression levels and activities in both male Sprague-Dawley rats and C57BL/6J mice. The inductive effect of Sf on mouse CYP2c needs further studies. In this report, only one lot of Sf extract was studied. Our previous mouse study demonstrated that the Sf extract prepared by different companies could show distinct chemical fingerprints and the presence of prenylated flavonoids was associated with the lack of induction of CYP3a in females. Thus, quality control of herbal preparations is a crucial factor in the use of herbal remedy in the clinical setting.

Theophylline has a narrow therapeutic index and CYP1A2 is the primary P450 involved in theophylline oxidation. Human CYP1A2 has the lowest \( K_m \) value and highest catalytic efficiency for theophylline oxidation; to a lesser extent, CYP2B, CYP2E1, and CYP3A also exhibit theophylline oxidation activity. The results of our rat study demonstrated that not only microsomal oxidation activities toward selective substrates of CYP1A, CYP2B, and CYP3A were increased by Sf, but also theophylline 1-demethylation, 3-demethylation and 8-oxidation activities were increased. The oral administration of Sf extract to rats stimulated the total metabolite formation rate of theophylline. This result revealed the potential for drug interaction of taking Sf extract before theophylline treatment.

The intravenous dosage of aminophylline is 6 mg/kg in humans and this dosage is equivalent to 4.8 mg/kg theophylline, which resulted in a plasma concentration of 5–20 \( \mu \)g/ml theophylline in patients. Due to the narrow therapeutic range of theophylline, changes in clearance of greater than 25% may have a clinical impact. Our pharmacokinetic analysis results were comparable to the parameters obtained from a blood microdialysis system in Sprague-Dawley rats intravenously treated with 2 mg/kg theophylline. Sf extract caused a 23% and 36% decrease of the AUC and AUUMC values of theophylline, respectively. The clearance of theophylline was significantly increased by 31% without affecting the distribution volume, distribution rate constants, or half-life of distribution. The half-lives of elimination and the terminal elimination phase as well as MRT were marginally decreased. The elimination rate constant was marginally increased but this increase did not show statistical significance between control and Sf-treated rats. The reasons for the lack of significance in the marginal alterations of elimination half-life and rate constant were not
clear. Possible factors include individual variation, a slight increase of distribution volume, and extrahepatic elimination. These pharmacokinetic changes together with the increased hepatic theophylline oxidation activity revealed that ingestion of Sf extract facilitated the excretion of theophylline in vivo. The increase of theophylline clearance by Sf extract was in the range of known pharmacokinetic interactions between theophylline and other drugs in humans.20)

In summary, our results demonstrated that ingestion of Sf extract could elevate the catalytic activities of multiple P450 forms in rat liver, including CYP1A2, CYP2B1/2, and CYP3A. Sf extract resulted in increases in theophylline 8-oxidation and N-demethylation activities. Although direct extrapolation from rat studies to the human response is difficult and the influence of Sf medical treatment in human drug metabolism needs further human study, our results revealed potential drug interaction with theophylline after taking Sf extract. Possible P450 induction-induced herb-drug interaction should be noted when patients previously used or concurrently use Sf extract and drug substrates of P450s, especially of CYP1A2. Optimal dosage and treatment time are important factors in P450 induction and can be crucial for preventing herb-drug interaction.

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References

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