Short Communication

LACK OF EVIDENCE FOR INDUCTION OF CYP2B1, CYP3A23, AND CYP1A2 GENE
EXPRESSION BY Panax ginseng AND Panax quinquefolius EXTRACTS IN ADULT RATS
AND PRIMARY CULTURES OF RAT HEPATOCYTES

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ABSTRACT:

Treatment of rats with a single oral dose (10–30 mg/kg) of a crude Panax ginseng extract of unknown ginsenoside content has been reported to modestly increase hepatic microsomal cytochrome P450-mediated aminopyrine N-demethylation activity. In the present study, we compared the effect of P. ginseng and Panax quinquefolius extracts on rat hepatic CYP2B1, CYP3A23, and CYP1A2 gene expression. Adult male Sprague-Dawley rats (250–275 g) received, by oral gavage or i.p., P. ginseng extract [4% (w/w) total ginsenosides; 30 or 100 mg/kg/day for 1 or 4 days], Panax quinquefolius extract [10% (w/w) total ginsenosides; 100 or 400 mg/kg/day for 21 consecutive days], or an equivalent volume (2 ml/kg) of the vehicle (0.9% NaCl or 0.3% carboxymethylcellulose) and were terminated 1 day after the last dose. P. ginseng and Panax quinquefolius extracts did not affect body weight gain, absolute or relative liver weight, hepatic CYP2B1, CYP3A23, or CYP1A2 mRNA expression, or microsomal CYP2B-mediated 7-benzyloxyresorufin O-dealkylation (BROD) or CYP1A-mediated 7-ethoxyresorufin O-dealkylation (EROD) activity. In contrast, results from positive control experiments indicated that phenobarbital increased CYP2B1 mRNA and BROD activity, dexamethasone increased CYP3A23 mRNA, and β-naphthoflavone increased CYP1A2 mRNA and EROD activity levels. Treatment of primary cultures of rat hepatocytes with either of the ginseng extracts [0.1–1000 μg/ml for 2 days] also did not affect CYP2B1 or CYP3A23 mRNA expression. Overall, our data indicate that P. ginseng and P. quinquefolius extracts do not increase rat hepatic CYP2B1, CYP3A23, or CYP1A2 gene expression.

For more than 3000 years, ginseng has been used in Asia for the treatment of various medical disorders. Since the 18th century, it has been one of the most commonly used herbal medicines in Europe and North America (Soldati, 2000). According to a recent survey, ginseng is the second most popular herbal medicine in the United States (Barnes et al., 2004). Several types of ginseng have been identified to date, including those in the Panax genus. The best characterized species in this genus are Panax ginseng C. A. Meyer (also known as Asian ginseng) and Panax quinquefolius L. (also known as North American ginseng) (Soldati, 2000).

The impetus for the present investigation is the paucity of information in the scientific literature on the effect of ginseng on hepatic expression of drug-metabolizing enzymes. In a previous study (Lee et al., 1987), the administration of a single oral dose (10, 20, or 30 mg/kg) of a crude P. ginseng extract to adult male rats resulted in a small but statistically significant increase (18–24%) in hepatic microsomal cytochrome P450 P450-mediated aminopyrine N-demethylation activity. In contrast, the same treatment did not increase S-9 aryl hydrocarbon hydroxylation activity in an assay that used benzo[a]pyrene as the substrate. The investigators used a crude extract of unknown ginsenoside content, and the effects on individual P450 enzymes were not studied. It is also not known whether another type of ginseng, such as P. quinquefolius, influences P450 expression.

CYP2B1 and CYP3A23 catalyze aminopyrine N-demethylation (Imoika et al., 1988), whereas CYP1A2 is a catalyst of aryl hydrocarbon hydroxylation (Ryan and Levin, 1990). Therefore, in the present study, we quantified hepatic CYP2B1, CYP3A23, and CYP1A2 expression in adult male rats administered P. ginseng or P. quinquefolius extract of known ginsenoside content. We also determined whether these extracts modulate P450 gene expression in primary cultures of rat hepatocytes.

Materials and Methods

Chemicals and Reagents. P. ginseng [GI15, lot 1254/485; 4% (w/w) total ginsenosides] and P. quinquefolius extracts [lot AGSP110200; 10% (w/w) total ginsenosides] were provided by Pharmaton S.A. (Bioggio, Switzerland) and the Canadian Phytopharmaceuticals Corp. (Richmond, BC, Canada), respectively. The amounts of ginsenosides Rb1, Rb2, Rc, Re, Rf, Rg1, and Rg2 present in the P. ginseng and P. quinquefolius extracts have been detailed elsewhere (Chang et al., 2002). 7-Ethoxyresorufin, 7-benzyloxyresorufin, 7-naphthoflavone, BROD, 7-benzyloxyresorufin O-dealkylation; DEX, dexamethasone; DMSO, dimethyl sulfoxide; EROD, 7-ethoxyresorufin O-dealkylation; P450, cytochrome P450; PB, phenobarbital; PCR, polymerase chain reaction.
β-naphthoflavone (BNF), dexamethasone (DEX), NADPH, bovine serum albumin, SYBR Green I, Williams’ medium E, fetal bovine serum, collagenase (type IV), trypsin inhibitor (type II-S), trypsin blue, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Phenobarbital (PB) was obtained from Wiler PCCA (London, ON, Canada). Human recombinant insulin, 10× Dulbecco’s phosphate-buffered saline, 10× Hanks’ balanced salt solution (without sodium bicarbonate and without phenol red), Liver Perfusion Media, Hepatocyte Wash Media, penicillin-streptomycin, and t-glutamine were obtained from Invitrogen (Burlington, ON, Canada). Matrigel basement membrane matrix was purchased from BD Biosciences Canada (Mississauga, ON, Canada). Percoll was bought from Amersham Biosciences, Inc. (Baie d’Urfe, QC, Canada). Bio-Rad Protein Assay Kit was bought from Bio-Rad (Hercules, CA). The sources of the polymerase chain reaction (PCR) primers and the reagents for isolation of total RNA, reverse transcription, quantification of total RNA and total cDNA concentrations, and real-time PCR have been detailed elsewhere (Chang et al., 2003).

Treatment of Animals. Adult male Sprague-Dawley rats (250–275 g) were purchased from Charles River Canada (Montreal, QC, Canada) and housed in our animal care facility, as described previously (Kuo et al., 2004). Rats were administered *P. ginseng* extract (30 or 100 mg/kg) or the vehicle (0.9% NaCl) by oral gavage as a single dose or as multiple doses (once daily for 4 consecutive days). In another experiment, rats were administered *P. quinquefolius* extract (100 or 400 mg/kg) or the vehicle (0.3% carboxymethylcellulose) by oral gavage once daily for 21 consecutive days. In positive control experiments, another group of rats was injected i.p. with BNF (40 mg/kg once daily for 3 consecutive days), PB (80 mg/kg once daily for 4 consecutive days), or DEX (100 mg/kg once daily for 3 consecutive days). All rats were terminated 1 day after the last dose and livers were excised.

Isolation, Culture, and Treatment of Rat Hepatocytes. Adult male rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Hepatocytes were isolated by a two-step collagenase perfusion method (Seglen, 1993), and cell viability was determined by trypan blue exclusion. Hepatocyte suspension was diluted to 10^7 cells/ml in serum-free, supplemented Williams’ medium E (which contained 1 μM insulin, 100 mM DEX, 2 mM t-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin), and a 3-ml volume was plated onto Matrigel-coated (Schuetz et al., 1988) culture dishes (60 × 15 mm; Permanax). Cells were then incubated for 4 h in a humidified 37°C incubator in the presence of 5% CO2 and 95% air. Subsequently, the medium was decanted to remove unattached cells, and hepatocytes were cultured in serum-free, supplemented Williams’ medium E. At 48 h after plating, cultured hepatocytes were treated for 48 h with *P. ginseng* extract (0.1–1000 μg/ml), *P. quinquefolius* extract (0.1–1000 μg/ml), or culture medium (vehicle control). In control experiments, cultured hepatocytes were treated with PB (100 μM), DEX (10 μM), or 0.1% DMSO (vehicle control). Culture medium was changed daily.

Isolation of Total RNA and Reverse Transcription. TRIzol was used to isolate total RNA from rat liver or cultured rat hepatocytes. The purity and integrity of each isolated RNA preparation was assessed as described previously (Cheung et al., 2004). Total cellular concentrations were determined using the RiboGreen RNA Quantitation Kit (Jones et al., 1997). Reverse transcription was performed with SuperScript II reverse transcriptase (Cheung et al., 2004), and total cDNA concentrations were quantified using the PicoGreen dsDNA Quantitation Kit (Singer et al., 1997).

**PCR Primers.** The specificity of the rat CYP1A2 (Borlak and Thum, 2001), CYP2B1 (Li and Kupfer, 1998), CYP3A23 (Mahnke et al., 1997), and cyclophilin (Morris and Davila, 1996) primers was confirmed by sequencing analysis of the purified amplicons (Cheung et al., 2004). The primers used to amplify CYP2B1 do not amplify CYP2B2, CYP2B3, or the CYP2B pseudogenes (Trottier et al., 1996), CYP2B14P and CYP2B16P. Similarly, the primers used to amplify CYP3A23 do not amplify CYP3A2, CYP3A9, or CYP3A18, and the primers used to amplify CYP1A2 do not amplify CYP1A1.

**Real-Time PCR Analysis.** Each 20-μl PCR reaction volume contained 1 unit of Platinum TaqDNA polymerase in 1× PCR reaction buffer [20 mM Tris-HCl (pH 8.4) and 50 mM KCl], 4 mM magnesium chloride, 1 ng of cDNA, 200 μM of deoxynucleoside-5’-triphosphate mix, 0.2 μM concentra-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP2B1 mRNA</th>
<th>CYP3A23 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. ginseng Extract (μg/ml)</td>
<td>fold-increase relative to control</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.78 ± 0.05</td>
<td>0.98 ± 0.14</td>
</tr>
<tr>
<td>1</td>
<td>0.86 ± 0.08</td>
<td>0.92 ± 0.02</td>
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<tr>
<td>10</td>
<td>0.95 ± 0.23</td>
<td>0.96 ± 0.21</td>
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<tr>
<td>100</td>
<td>0.93 ± 0.27</td>
<td>0.79 ± 0.01</td>
</tr>
<tr>
<td>1000</td>
<td>0.51 ± 0.25</td>
<td>0.31 ± 0.10*</td>
</tr>
<tr>
<td>P. quinquefolius Extract (μg/ml)</td>
<td>fold-increase relative to control</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.84 ± 0.11</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>1</td>
<td>0.78 ± 0.07</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>0.88 ± 0.14</td>
<td>0.72 ± 0.06</td>
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<tr>
<td>100</td>
<td>1.16 ± 0.26</td>
<td>1.00 ± 0.20</td>
</tr>
<tr>
<td>1000</td>
<td>0.47 ± 0.11*</td>
<td>0.87 ± 0.14</td>
</tr>
<tr>
<td>PB (100 μM)</td>
<td>49 ± 22*</td>
<td>not determined</td>
</tr>
<tr>
<td>DEX (10 μM)</td>
<td>not determined</td>
<td>41 ± 2*</td>
</tr>
</tbody>
</table>

* Significantly different from the corresponding vehicle-treated control group (p < 0.05).
tion of each of the forward and reverse primers, 0.25 mg/ml bovine serum albumin, and 2 μl of a 3.5× SYBR Green I solution. The cycling conditions for real-time cDNA amplification (LightCycler; Roche Diagnostics, Mannheim, Germany) were 95°C for 1 s (denaturation), 60°C for 6 s (annealing), and 72°C for 10 s (extension) for CYP1A2; 95°C for 1 s, 56°C for 6 s, and 72°C for 23 s for CYP2B1; 95°C for 1 s, 60°C for 6 s, and 72°C for 14 s for CYP3A23; and 95°C for 1 s, 56°C for 6 s, and 72°C for 12 s for cyclophilin. In all cases, the initial denaturation was performed at 95°C for 5 min. The real-time PCR assays had a bias of less than 5%, and precision (% CV) was less than 19%. The intraday variability ranged from 3 to 8%, and the interday variability was from 8 to 30%.

Preparation of Microsomes, Protein Assay, and 7-Alkoxysresorufin O-Dealkylation Assays. Microsomes were isolated by differential ultracentrifugation (Lu and Levin, 1972). Protein concentrations were determined using the Bio-Rad Protein Assay Kit. EROD and BROD activities were determined as described previously (Kuo et al., 2004).

Statistics. The significance of the difference between the means was assessed by one-way analysis of variance and, where appropriate, was followed by the Student Newman-Keuls test (SigmaStat software program; SPSS Inc., Chicago, IL). The level of significance was set a priori at p < 0.05.

Results and Discussion

In the present study, the oral administration of a single dose of P. ginseng extract of known ginsenoside content (Chang et al., 2002) at a dosage of 30 mg/kg or 100 mg/kg to adult male rats did not affect body weight gain or liver weight (data not shown). The 30 mg/kg dosage was chosen because a previous study indicated that treatment of adult male rats with a single dose at 10 to 30 mg/kg resulted in a modest increase in P450-mediated enzyme activity (Lee et al., 1987). However, our real-time PCR analysis showed that P. ginseng extract did not modulate CYP2B1 (Fig. 1A), CYP3A23 (Fig. 1B), or CYP1A2 (Fig. 1C) mRNA expression. The same conclusions were drawn regardless of whether the gene expression data were normalized to the amount of total double-stranded DNA (e.g., Fig. 1, A–C) or to cyclophilin mRNA levels (data not shown). There was also a lack of an effect on hepatic microsomal CYP1A-mediated EROD and CYP2B-mediated BROD activities (data not shown). Similarly, oral or i.p. administration of P. ginseng extract at a dosage of 100 mg/kg once daily for 4 consecutive days did not influence hepatic CYP2B1, CYP3A23, or CYP1A2 gene expression (data not shown). In contrast, positive control experiments indicated that PB increased hepatic CYP2B1 mRNA expression (47-fold; Fig. 1A) and microsomal BROD activity (37-fold), DEX increased hepatic CYP3A23 mRNA expression (75-fold; Fig. 1B), and BNF increased hepatic CYP1A2 mRNA expression (20-fold; Fig. 1C) and microsomal EROD activity (19-fold).

Our data obtained with the in vivo administration of P. ginseng are consistent with those reported in recent clinical studies. In an open-label, randomized study (Gurlay et al., 2002) performed in healthy volunteers (six males and six females), the oral ingestion of P. ginseng (500 mg three times daily for 28 days, and formulated to contain 5% total ginsenosides) did not modify the group mean 1-h serum ratio of 1-hydroxymidazolam/midazolam, which is used as an in vivo index of CYP3A4 activity (Thummel et al., 1994), or the 6-h serum ratio of paraxanthine/caffeine, which is used as an in vivo index of CYP1A2 activity (Fuhr and Rost, 1994). In another clinical study (Anderson et al., 2003), the oral ingestion of P. ginseng (Ginsana, 100 mg twice daily for 14 days, and formulated to contain 4% total ginsenosides) by 12 adult female volunteers did not affect the urinary 6β-hydroxy cortisol/cortisol ratio, which is another in vivo index of CYP3A4 expression (Galteau and Shamsa, 2003).

P. ginseng and P. quinquefolius differ chemically from each other in several ways: 1) ginsenoside Rf is present in P. ginseng but absent in P. quinquefolius (Li et al., 2000); 2) 24-β-pseudoginsenoside Rf₁₁ is absent in P. ginseng but present in P. quinquefolius (Li et al., 2000); 3) the ratio of ginsenoside Rb₁ to ginsenoside Rb₂ is greater in P. quinquefolius than in P. ginseng (Li et al., 2000); and 4) the ratio of ginsenoside Rg₁ to ginsenoside Rb₁ is less in P. quinquefolius than in P. ginseng (Ji et al., 2001). Therefore, not surprisingly, differences have been reported in the magnitude of the effects produced by these two types of ginseng. For example, in our recent study (Chang et al., 2002), P. quinquefolius extract was 45-fold more potent than P. ginseng extract in the in vitro inhibition of human CYP1A2 catalytic activity, as determined by a comparison of the apparent Kᵢ values. In the present investigation, rats were treated orally with P. quinquefolius extract at a dosage of 100 or 400 mg/kg once daily for 21 consecutive days. This dosage regimen was chosen because a previous study showed that P. quinquefolius extract administered at a dosage of 100 mg/kg once daily for 14 or 28 days was effective in modulating specific biological responses in rats (Murphy et al., 1998). Our results indicated that P. quinquefolius extract did not affect body weight gain or liver weight (data not shown). It also did not increase hepatic CYP2B1, CYP3A23, or CYP1A2 gene expression, or hepatic microsomal CYP2B-catalyzed BROD activity or CYP1A1-catalyzed EROD activity (data not shown).

To determine directly whether ginseng extract is capable of increasing CYP2B1 and CYP3A23 gene expression, primary cultures of rat hepatocytes were treated for 48 h with P. ginseng or P. quinquefolius extract at a concentration of 0.1, 1, 10, 100, or 1000 μg/ml. As indicated in Table 1, these extracts did not increase CYP2B1 or CYP3A23 mRNA levels, whereas control experiments showed PB induction of CYP2B1 mRNA (49-fold) and DEX induction of CYP3A23 mRNA (41-fold) in cultured rat hepatocytes. Overall, the in vitro data indicate that the lack of an effect by the in vivo administration of the ginseng extracts was likely not due to inadequate bioavailability.

In summary, the present study provides the first demonstration in rats and primary cultures of rat hepatocytes that P. ginseng and P. quinquefolius extracts of known ginsenoside content do not induce CYP2B1, CYP3A23, or CYP1A2 gene expression.

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