Cytochrome P450 Dysregulations in Thioacetamide-Induced Liver Cirrhosis in Rats and the Counteracting Effects of Hepatoprotective Agents

Yuan Xie, Guangji Wang, Hong Wang, Xilin Yao, Shan Jiang, An Kang, Fang Zhou, Tong Xie, and Haiping Hao

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ABSTRACT:

Dysregulations of cytochromes P450 (P450s) under liver injury have been extensively studied. However, little is known about the possible reversing effects of hepatoprotective agents, the understanding of which is of great importance in guiding clinical dosage adjustment for patients with liver injury. This study aims to investigate the dysregulation patterns of major P450s in thioacetamide (TAA)-induced liver cirrhosis in rats and the potential counteracting effects of hepatoprotective agents schisandra lignans extract (SLE) and dimethyl diphenyl bicarboxylate (DDB). TAA intoxications for 6 weeks induced apparent liver injury and dramatically reduced the hepatic protein expressions of CYP1A2, CYP2C6, CYP2E1, and CYP3A2 to 18, 71, 30, and 21% of that in the normal control, respectively. Both SLE and DDB treatments could significantly reverse the TAA-induced loss of P450 protein levels, which may be ascribed to their hepatoprotective effects and direct P450-inducing effects that have been confirmed in healthy rats. However, the recovery of enzyme activities of most P450s by SLE and DDB treatment was less evident than that for the protein expression levels. TAA exhibited NADPH-, time-, and concentration-dependent inactivating effects on all of the four major P450 isoforms; both DDB and GSH showed little effects on counteracting such an inactivation efficacy. These findings provided a good explanation on the disproportional effects of hepatoprotective agents in recovering the protein levels and enzyme activities of TAA-induced dysregulated P450s.

Introduction

Thioacetamide (TAA), a potent selective hepatotoxin, is well known to induce both acute and chronic hepatic failure (Steib et al., 2010; Zaldivar et al., 2010; Ishikawa et al., 2011). Prolonged exposure to TAA always results in bile duct proliferation and liver cirrhosis histologically similar to that in human viral hepatitis infection (Hunter et al., 1977; Ledda-Columbano et al., 1991; Yeh et al., 2004). Because of this virtue, TAA has been widely applied to develop animal models of liver fibrosis and/or cirrhosis mimicking human nonbiliary liver diseases. From these animal models, many important pathological processes and involved mechanisms of liver fibrosis/cirrhosis had been disclosed, contributing to the development of novel diagnostic and therapeutic approaches and hepatoprotective drugs.

Human liver diseases caused by various factors usually lead to the dysregulations of most drug-metabolizing enzymes (DMEs), especially cytochromes P450 (P450s), which necessitates the dosage adjustment of therapeutic drugs in patients with severe liver diseases. Unfortunately, the present understanding of this critical issue is extremely limited. Increased expression of CYP2E1 and CYP2A5 was observed in TAA-induced liver injury models (Camus-Randon et al., 1996; Avni et al., 2003); a previous report showed that nicotine metabolism was significantly decreased in the TAA-induced fibrotic rat livers, associated with the down-regulated protein levels of most P450s (Nakajima et al., 1998). However, TAA-induced potential regulation of enzyme activity of P450s remains unclear. TAA is a thiono-sulfur-containing compound and is readily bioactivated by CYP2E1 and flavin-containing monooxygenase to produce acetamide and thioacetamide-S-dioxide. These reactive metabolites may covalently bind to various proteins, which is indeed the major mechanism of TAA on causing liver toxicity. On the basis of these characteristics,

ABBRVIATIONS: TAA, thioacetamide; DME, drug-metabolizing enzyme; P450, cytochrome P450; SLE, schisandra lignans extract; DDB, dimethyl diphenyl bicarboxylate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; Tbil, total bilirubin; RLM, rat liver microsomes; PVDF, polyvinylidene fluoride; UGT, UDP-glucuronosyltransferase.
we hypothesized that TAA intoxication may lead to both the translational dysregulations and the direct enzyme inactivation of P450s. To test this hypothesis, both protein levels and enzyme activities of major P450s were determined in the chronic TAA-treated rat livers. Potential inactivating effects of TAA on P450s were performed in the in vitro liver microsomal incubation systems.

In addition, it should be noted that the patients with liver diseases are usually inclined to consume hepatoprotective agents. Therefore, it is important to understand whether such hepatoprotective agents can influence the dysregulated P450s in injured livers, which is of critical concern for adjusting the dosage regimen of coadministered drugs. Current understanding of such an important issue is extremely limited. We have found that the hepatoprotective agent schisandra lignans extract (SLE) was effective at protecting the liver from CCl4-induced damage and restoring the pharmacokinetic characteristics of its lignans components, indicating the recovery of some P450s (Xie et al., 2010). Based on a TAA-induced liver cirrhosis rat model that better mimics human liver cirrhosis, the present study was further designed to determine whether SLE treatment would be effective at recovering TAA-induced dysregulations of major P450s.

Materials and Methods

**Chemicals and Reagents.** TAA was obtained from Jiahui Medicine Chemical Company LLC (Anhui, China). Dimethyl diphenyl bicarboxylate (DDB) was purchased from Hangzhou Dengyun Pharm and Tech Co., Ltd Pharmacy (Zhejiang, China). The ethanol extract of *Schisandra chinensis* Baill (SLE) was purchased from Nanjing Qingze Pharmaceuticals Company (Jiangsu, China). Midazolam, chlorozoxazone, diclofenac, phenacetin, and acetaminophen were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 4′-Hydroxydiclofenac, 4-hydroxy-7-methylcoumarin, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). High-performance liquid chromatography-grade acetonitrile, and glucose-6-phosphate, NADP+ and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). High-performance liquid chromatography-grade acetonitrile, and methanol, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore Corporation, Billerica, MA). Radio immunoprecipitation assay analysis buffer, phenylmethylsulfonyl fluoride, and SDS-polyacrylamide gel electrophoresis sample-loading buffer were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Polyvinylidene difluoride (PVDF) membranes were obtained from Millipore (Shanghai, China). Rabbit anti-rat CYP3A2 polyclonal antibody, rabbit anti-rat CYP2E1 polyclonal antibody, and mouse anti-rat CYP1A2 polyclonal antibody were from Millipore Bioscience Research Reagents (Temecula, CA). Mouse anti-rat CYP2C6 polyclonal antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Mouse anti-rat β-actin polyclonal antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG and goat anti-mouse IgG were purchased from Boster Biological Technology, Ltd. (Wuhan, China). An enhanced chemiluminescence kit was purchased from Thermo Fisher Scientific (Waltham, MA).

**Animals and Experimental Design.** Male Sprague-Dawley rats (180–220 g) were obtained from the Academy of Military Medical Sciences (Beijing, China). All rats were maintained in an air-conditioned animal quarter at a temperature of 25 ± 2°C and a relative humidity of 50 ± 10%. Water and food were allowed ad libitum. The animals were acclimatized to the facilities for a week and then fasted with free access to water for 12 h before each experiment. All animal experimental procedures were approved by the Animal Care and Use Committee of China Pharmaceutical University and have been performed in accordance with the Declaration of Helsinki. Rats were randomly divided into five groups with six animals in each group. TAA was intraperitoneally administered (200 mg/kg, 3% in saline, twice a week for 6 weeks) to all groups of rats except the normal control group, which received normal saline injection. From the beginning of the 5th week, rats were intragastrically treated with sodium carboxymethyl cellulose suspension (group I and II), SLE (100 mg/kg per day, group III), SLE (400 mg/kg per day, group IV), or DDB (200 mg/kg per day, group V) for 2 weeks. To determine the effects of SLE and DDB on regulating P450s in healthy rats, three groups of healthy rats received intragastric administration of SLE (100 mg/kg per day), SLE (400 mg/kg per day), or DDB (200 mg/kg per day) for 2 weeks. At the end of treatments, blood samples were collected; the rats were then euthanized, and the liver samples were immediately removed and frozen in liquid nitrogen.

**Blood Biochemical Analysis and Histopathological Study.** Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), and total bilirubin (Tbil) were determined by an automatic blood biochemical analyzer (LX20; Beckman Coulter, Inc., Fullerton, CA). Slices of the same part of the livers were cut off and fixed in phosphate-buffered 10% formaldehyde solution and then were embedded in paraffin wax. Sections of liver tissue (6 mm × 5 μm) were cut and stained with hematoxylin and eosin and were examined for histopathological changes under the microscope (Olympus BH2; Olympus, Tokyo, Japan). The images were taken using Nicon CoolPix 990 camera (Nikon, Melville, NY) at original magnification of 100× and 200×.
determined using the specific substrate for each of the isozymes. Clint was calculated by
1–400 desribed previously with minor modifications (Qiu et al., 2008). A 200-
6-hydroxylation for CYP2E1, and midazolam 4-hydroxylation for CYP3A2, as

tion for CYP1A2, diclofenac 4-hydroxylation for CYP2C6, chlorzoxazone
analysis;
CYP1A2, CYP2C6, CYP3A2, and CYP2E1 were determined via Western blot
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FIG. 2. Western blot analyses of major P450s in rat liver samples. Rats were treated
with TAA for 4 weeks and then were cotreated with or without SLE or DDB for
another 2 weeks. Liver microsomes were prepared, and the enzyme kinetics of P450s was
enlarged using a two-step procedure. Primary incubations were performed in a
media containing 0.5 mM NADPH, 5 mg/ml RLM, and various concentrations of
TAA in 100 mM phosphate buffer (pH 7.4) in a final volume of 200 μl. At
various time points (0, 5, 10, 15, and 30 min), 20 μl of the primary
media was removed and added into a secondary reaction media containing
specific P450 substrate, NADPH-regenerating system, and buffer as usual. All
experimental procedures of the secondary incubations were the same as those
described above for the enzyme activity assay of P450s. All incubations were
performed in triplicates.

Western Blot Analyses. Protein levels of the major rat P450 isoforms were
determined by a Western blot analysis. Liver protein samples (10 μg protein/ lane) were separated by SDS-polyacrylamide gel electrophoresis with an 8%
polyacrylamide gel and were transferred to a PVDF membrane by electroblot-
ing. The PVDF sheets were blocked in 5% nonfat dry milk in Tris-buffered
saline/0.05% Tween 20 (TBST) at 37°C for 1 h. The blots were incubated with
primary antibodies diluted in 5% nonfat dry milk in TBST overnight at 4°C
followed by incubation with goat anti-rabbit (CYP2E1 and CYP3A2) or goat
anti-mouse (CYP1A2 and CYP2C6) secondary antibodies for 1 h. The primary
antibodies against CYP1A2, CYP3A2, CYP2C6, and CYP2E1 were diluted
1:1500, 1:1500, 1:500, 1:1000, and 1:1000, respectively. For reference, β-actin
was detected using a polyclonal antibody (1:200) for 1 h and then was
incubated with secondary antibody horseradish peroxidase-conjugated goat
anti-rabbit IgG (1:1500) for 1 h at 37°C. The immunoblots were developed
with the enhanced chemiluminescence detection method with reagents from
Biorad Laboratories Inc. (Minneapolis, MN). Proteins were detected by
enhanced chemiluminescence kit. Quantification of relative P450 levels was
performed by laser densitometry of the X-ray films. The density of the
immunoreactive bands was analyzed using ImageJ software (National Insti-
tutes of Health, Bethesda, MD).

Statistical Analysis. The values of the experimental data were expressed as
mean ± S.D. Statistical significances were evaluated using one-way analysis
of variance, followed by the post hoc least significant difference method for
multiple comparisons. The difference was considered significant when p < 0.05.

Results

TAA-Induced Liver Cirrhosis and Hepatoprotective Effects of
SLE and DDB. We have reported previously that TAA intoxication
for 8 weeks caused severe liver damage in rats (Hao et al., 2011). The
present study showed that TAA intoxication for 6 weeks could also
induce apparent hepatic injury, as evidenced by the significantly
enhanced levels of serum ALT, AST, ALP, and Tbil (p < 0.01). SLE
and DDB treatment for 2 weeks could partially reverse TAA-induced
alterations of serum ALT, AST, and Tbil levels, supporting the
hepatoprotective effects of both SLE and DDB (Supplemental Fig. 1).
TAA induced extensive changes in liver morphology including ste-
atosis, inflammation, fibrosis, centrilobular necrosis, and scattered

cates. The intrinsic clearance (Clint) values were determined by nonlinear
regression analysis of the enzyme activity-substrate concentration data using
the Michaelis-Menten model.

Mechanism-based inactivations of TAA on microsomal P450s were as-
sewed using a two-step procedure. Primary incubations were performed in
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TAA induced extensive changes in liver morphology including ste-
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![Table 1](https://example.com/table1.png)

**Table 1**

Clint of liver microsomal P450s in normal, TAA-induced hepatic injury rats treated with or without SLE and DDB

<table>
<thead>
<tr>
<th>Group</th>
<th>CYP1A2</th>
<th>CYP2C6</th>
<th>CYP3A2</th>
<th>CYP2E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3741</td>
<td>1.00</td>
<td>0.0079</td>
<td>1.00</td>
</tr>
<tr>
<td>TAA</td>
<td>0.1847</td>
<td>0.49</td>
<td>0.0026</td>
<td>0.33</td>
</tr>
<tr>
<td>SLE (100 mg/kg) + TAA</td>
<td>0.2351</td>
<td>0.63</td>
<td>0.0029</td>
<td>0.36</td>
</tr>
<tr>
<td>SLE (400 mg/kg) + TAA</td>
<td>0.3185</td>
<td>0.85</td>
<td>0.0029</td>
<td>0.36</td>
</tr>
<tr>
<td>DDB (200 mg/kg) + TAA</td>
<td>0.2930</td>
<td>0.78</td>
<td>0.0028</td>
<td>0.35</td>
</tr>
</tbody>
</table>

**Notes:**
- †, p < 0.05, versus normal group
- ††, p < 0.01, versus TAA group
- †‡, p < 0.001, versus TAA group

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**Fig. 2.** Western blot analyses of major P450s in rat liver samples. Rats were treated
with TAA for 4 weeks and then were cotreated with or without SLE or DDB for
another 2 weeks. Liver microsomes were prepared, and the enzyme kinetics of P450s was
determined by a Western blot analysis.
lymphomononuclear infiltration in hepatic parenchyma. Treatment with SLE exhibited dose-dependent reversal effects on TAA-induced liver fibrosis, characterized with decreased necrotic zones and hepatocellular degeneration (Fig. 1).

**Expression of Hepatic P450s in TAA-Induced Cirrhotic Livers.** Hepatic P450 protein levels were determined by Western blot analyses. TAA intoxication significantly decreased CYP1A2, CYP2C6, CYP2E1, and CYP3A2 protein levels to 18, 71, 30, and 21% of that of the normal control, respectively. SLE and DDB treatment markedly attenuated TAA intoxication, causing down-regulation of the protein levels of all these P450s, especially for CYP3A2 and CYP2E1 (Fig. 2). It was noted that the protein levels of some P450s in SLE- and DDB-treated rats were even higher than those in the normal control group; CYP3A2 protein levels in SLE (100 and 400 mg/kg) and DDB (200 mg/kg)-treated rats were 1.09-fold, 1.37-fold, and 1.56-fold of that in the normal control; CYP2E1 protein level in DDB-treated rats was 2.26-fold of that in the normal control (Fig. 2).

**Activity of Hepatic P450s in TAA-Induced Cirrhotic Livers.** Liver microsomes were prepared from each group of rats to test the enzyme activities of P450s using the typical probe substrate approach. Results are shown in Table 1. As evidenced from Cl_mic values, TAA intoxication markedly decreased the activity of CYP1A2, CYP2C6, CYP3A2, and CYP2E1 to 49, 33, 69, and 43% of that in the normal control, respectively. SLE and DDB treatment partially reversed TAA-induced loss of CYP1A2, CYP2E1, and CYP3A4 activity. In contrast, CYP2C6 activity was less affected by SLE and DDB treatment. Correlation analysis showed that the activity and protein expression were consistent for CYP1A2 and CYP3A2, but not for CYP2E1 and CYP2C6 (Fig. 3).

**Activity and Expression of Hepatic P450s in Healthy Rats Treated with SLE/DDB.** To clarify the effects of SLE and DDB on regulating normal rat liver P450s, rats were treated with SLE and DDB for 2 weeks, and the activity and expression of CYP1A2, CYP2C6, CYP3A2, and CYP2E1 were determined. As shown in Table 2, SLE and DDB showed inhibitory effects on CYP1A2, whereas both SLE and DDB largely enhanced the activity of CYP2C6, CYP3A2, and CYP2E1. Consistently, the results of Western blot analysis showed the decrease of CYP1A2 but the increase of CYP2C6, CYP3A2, and CYP2E1 protein levels in the livers of SLE- and DDB-treated healthy rats (Fig. 4).

**Mechanism-Based Inhibitory Effects of TAA on P450s.** In view of the bioactivation characteristics of TAA, we further investigated the potential mechanism-based inhibitory effect of TAA on P450s. TAA (0, 5, 50, 200, and 400 μM) was incubated with normal RLMs by adding the NADPH-regenerating system; the remaining enzyme activity at indicated time points was determined by a typical dilution approach (Brady et al., 1987; Li et al., 2009). Appropriate controls, lacking TAA, were included to ensure that the loss of activity was not due to TAA irrelevant inactivation. Without the addition of NADPH, TAA has little effect on inactivating P450s (data not shown). TAA inhibited CYP1A2, CYP2C6, CYP3A2, and CYP2E1 activity in a concentration- and time-dependent manner when NADPH was included in the incubations (Fig. 5). The observed first-order rate constants (k_inact) for the inactivation of P450s by individual concentrations of TAA were obtained from the slope of individual lines. These slopes were fit to a Kitz-Wilson plot, which is shown in the inset of Fig. 5. The calculated K_I and k_inact values are shown in Table 3. As evidenced from the k_inact/K_I values, it seems that

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>CYP1A2</th>
<th>CYP2C6</th>
<th>CYP3A2</th>
<th>CYP2E1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl_int</td>
<td>Times of Control</td>
<td>Cl_int</td>
<td>Times of Control</td>
</tr>
<tr>
<td></td>
<td>μl/min/mg</td>
<td>protein</td>
<td>μl/min/mg</td>
<td>protein</td>
</tr>
<tr>
<td>Control</td>
<td>0.3741</td>
<td>1.00</td>
<td>0.0079</td>
<td>1.00</td>
</tr>
<tr>
<td>SLE (100 mg/kg)</td>
<td>0.2728</td>
<td>0.73</td>
<td>0.0142</td>
<td>1.79</td>
</tr>
<tr>
<td>SLE (400 mg/kg)</td>
<td>0.2027</td>
<td>0.54</td>
<td>0.0252</td>
<td>3.18</td>
</tr>
<tr>
<td>DDB (200 mg/kg)</td>
<td>0.3502</td>
<td>0.94</td>
<td>0.0216</td>
<td>2.72</td>
</tr>
</tbody>
</table>
CYP3A2 and CYP2C6 were more susceptible than CYP1A2 and CYP2E1 to the TAA-induced mechanism-based enzyme inactivation’s. The addition of DDB and reduced GSH to the primary incubation systems had little effect on protecting against TAA-induced enzyme inactivations of P450s (Fig. 6).

**Discussion**

Intensive understanding of P450s dysregulation patterns in liver injury is of critical concern because of its significance in guiding clinical dosage regimen adjustment in patients with liver diseases. Considering that patients with liver diseases are inclined to consume hepatoprotective agents, it is important to understand the mixed effects of pathological factors and hepatoprotective agents on regulating P450s. The present study contributes to confirm that the protein levels and enzyme activities of P450s were decreased in TAA-induced rat cirrhotic livers. Upon NADPH-dependent bioactivation, TAA was able to inactivate enzyme activities of P450s in a time- and concentration-dependent manner. Treatment with hepatoprotective agents SLE and DDB could partially restore TAA-induced protein loss of P450s but had little direct effect on antagonizing enzyme-inactivating effects of TAA.

Dosage regimen adjustment for many therapeutic drugs is necessary for patients with severe liver diseases, because of the dysregulations of various DMEs in damaged livers. However, it remains a difficult issue for designing an ideal dosage adjustment regimen because various DMEs are not altered in a uniform extent in the damaged livers. The case may be further complicated by the concomitant ingestion of hepatoprotective agents that may directly or indirectly regulate the protein expression and/or enzyme activities of DMEs. Taking SLE and DDB as examples, the present study highlighted the importance of studying the regulation of DMEs by hepatoprotective agents in damaged livers. In accordance with a previous report (Nakajima et al., 1998), we confirmed that the protein levels of all the four major P450 isozymes were significantly reduced by chronic TAA intoxication, albeit to a varying extent for different isozymes. Results revealed that the hepatoprotective agents SLE and DDB largely attenuated TAA-induced protein loss of most P450 isozymes. The protein levels of CYP2E1 and CYP3A2 in TAA-intoxicated rats with SLE and DDB treatment were even higher than those in the normal control rats. These results suggest that SLE and DDB may have direct inducing effects on P450s. For this consideration, both protein levels and enzyme activities of major P450s were determined in the healthy rats treated with SLE and DDB for 2 weeks.

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**FIG. 4.** Western blot analyses of P450s in the liver samples of healthy rats treated with SLE and DDB. Rats were intragastrically treated with SLE and DDB once a day for 2 weeks. Protein expression levels of CYP1A2, CYP2C6, CYP3A2, and CYP2E1 were determined via Western blot analysis; β-actin was used as an internal standard to normalize all samples.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (SLE(400mg/kg))</th>
<th>SLE(100mg/kg)</th>
<th>DDB(200mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CYP3A2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CYP2E1</td>
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</table>

**FIG. 5.** Time- and concentration-dependent inactivation of P450s by TAA. TAA (0, 5, 50, 200, and 400 μM) was incubated with normal RLMs by adding the NADPH-regenerating system; the remaining enzyme activity was then determined by a typical dilution method. The $k_{obs}$ was obtained from the slope of the individual lines, and these slopes were fit to a Kitz-Wilson plot (inset). A, CYP2C6; B, CYP1A2; C, CYP3A2; D, CYP2E1.
Results showed that SLE and DDB treatment could significantly enhance the protein expressions and enzyme activities of most P450 isozymes except CYP1A2, for which an inhibitory effect was observed instead. Previously, we had also confirmed that after long-term consumption, SLE could induce the protein expression of CYP3A2 in both the rat intestine and the rat liver (Lai et al., 2009). Schisandra had been proven to be a strong inducer of CYP3A4 and CYP2C9 through pregnane X receptor in normal hepatocyte cultures (Mu et al., 2006). Together, these results indicate that SLE and DDB have direct inducing effects on P450s, which provides a good explanation about why the protein expression levels of CYP3A2 and CYP2E1 in TAA-intoxicated and SLE/DDB-treated rats are up-regulated to a level higher than that in the normal control. Although SLE and DDB show inhibitory effects in CYP1A2, the loss of CYP1A2 in TAA-damaged livers could be largely restored by treatment with hepatoprotective agents, suggesting a differential regulating effect in between healthy and damaged livers by hepatoprotective agents. These results in combination suggest that the restoration of P450s in damaged livers by hepatoprotective agents may be ascribed to both the direct enzyme-inducing effects and the hepatoprotective-driving consequent effects.

Using the typical probe substrate approach, we found that the enzyme activities of all the major P450s were significantly decreased in TAA-induced cirrhotic livers. We were surprised to find that the enzyme activities of most P450s were not restored in proportion to the protein levels by treatment of hepatoprotective agents. In view that TAA can be bioactivated by CYP2E1 to produce highly reactive metabolite di-S-oxide (Kang et al., 2008), we hypothesized that TAA upon bioactivation may inactivate P450s. Our results showed that TAA exhibited both time- and concentration-dependent inhibitory effect on all the four P450 isozymes when NADPH was added to initiate bioactivations. In contrast, TAA had little effect on inhibiting P450s when NADPH was omitted from the incubation systems. In accordance, we have recently found that TAA could also inactivate UDP-glucuronosyltransferases (UGTs) (Hao et al., 2011). In addition, DDB and GSH could not counteract such an inactivation; this result may suggest that the oxidative stress produced from the procedure of TAA bioactivation has little effect on inactivating P450s. It is thus understandable why the recovery effects of hepatoprotective agents in these enzyme activities are less evident than that in the protein levels of P450s in TAA-intoxicated rats. We have shown that both SLE and DDB had little effect on inhibiting TAA metabolism. Furthermore, the results from the present study suggest that SLE and DDB could not inhibit CYP2E1, which was identified as the major enzyme responsible for TAA bioactivation and hepatotoxicity. Together, our results suggest the hepatoprotective effects of SLE and DDB are not likely resourced from the direct influence on the metabolic bioactivation of TAA. In combination with our previous report (Hao et al., 2011), our results provide novel evidence supporting the previous assumption that TAA hepatotoxicity is mainly caused by TAA’s reactive metabolites on covalently modifying macromolecules (Chieli and Malvaldi, 1984). However, it is important to note that the TAA chronic intoxication leads to a differential regulating mode between P450s and

![Graph 1](image1)

**FIG. 6.** The effects of DDB and GSH on TAA-induced inactivation of P450s. TAA (50 μM) was incubated with normal RLMs containing a NADPH-regenerating system with or without the addition of DDB (2, 10, and 50 μM) or GSH (0.2, 1, 5 mM); the remaining enzyme activity of CYP2C6, CYP1A2, CYP3A2, and CYP2E1 was then determined by a typical dilution method. *p ≤ 0.05 versus control.

<table>
<thead>
<tr>
<th>P450 Isozymes</th>
<th>K&lt;sub&gt;i&lt;/sub&gt;</th>
<th>k&lt;sub&gt;inact&lt;/sub&gt;</th>
<th>k&lt;sub&gt;inact&lt;/sub&gt;/K&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>10.558 μM</td>
<td>0.018 min⁻¹</td>
<td>0.0017 min⁻¹/μM⁻¹</td>
</tr>
<tr>
<td>CYP2C6</td>
<td>22.812 μM</td>
<td>0.074 min⁻¹</td>
<td>0.0033 min⁻¹/μM⁻¹</td>
</tr>
<tr>
<td>CYP3A2</td>
<td>58.914 μM</td>
<td>0.056 min⁻¹</td>
<td>0.0010 min⁻¹/μM⁻¹</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>6.700 μM</td>
<td>0.026 min⁻¹</td>
<td>0.0039 min⁻¹/μM⁻¹</td>
</tr>
</tbody>
</table>
UGTs, although both can be inactivated by TAA bioactivation. In addition, the effects of hepatoprotective agents on regulating P450s and UGTs are also different. It may deserve further research to delineate the direct effects and underlying mechanisms of TAA’s reactive metabolites on inactivating various DMEs, thus providing strong scientific evidence on explaining the diverse regulating patterns of DMEs in TAA-induced cirrhotic livers.

On the basis of the TAA-induced liver fibrosis/cirrhosis model and the study of SLE and DDB, the present study highlights the importance of investigating the mixed effects of hepatic pathological factors and treatment by hepatoprotective agents on regulating P450s. Our results show that TAA intoxication causes a tremendous loss of P450s, whereas the treatments with the hepatoprotective agents SLE and DDB attenuate the protein loss of P450s. SLE and DDB have a direct inducing effect on most of the P450 isoforms except CYP1A2 in healthy rats. TAA exhibits a NADPH-, time-, and concentration-dependent inactivating effect on all of the four P450 isoforms, and both DDB and GSH have little effect on counteracting such an inactivation effect, providing a good explanation about the disproportional recovery of protein levels and enzyme activities of P450s by treatment with hepatoprotective agents. Hopefully, our results will stimulate wide interest in investigating the effects of various hepatoprotective agents on regulating DMEs in injured livers, the understanding of which is pivotal in guiding the design of a dosage adjustment regimen for patients with severe liver diseases.

Authorship Contributions

Participated in research design: Y. Xie, G. Wang, and Hao.

Conducted experiments: Y. Xie, H. Wang, Jiang, Yao, Kang, Zhou, T. Xie, and Hao.

Performed data analysis: Y. Xie and Hao.

Wrote or contributed to the writing of the manuscript: Y. Xie and Hao.

References


Cytochrome P450 dysregulations in thioacetamide induced liver cirrhosis in rats and the counteracting effects of hepatoprotective agents

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Drug Metabolism and Disposition

Supplemental Fig.1. Serum biochemical parameters of liver function after 6 weeks of TAA intoxication. Rats were treated with TAA for 4 weeks and then co-treated with or without SLE/DDB for another 2 weeks. Data are expressed as percentage (%) of control values (mean ± S.D., n=6). * p≤0.05, ** p≤0.01, vs. normal control; † p≤0.05, †† p≤0.01, vs. TAA control.