Reversing effects of silybin on TAA-induced hepatic CYP3A dysfunction through PXR regulation

XIE Yuan 1, HAO Hai-Ping 2, WANG Hong 2, WANG Zhao-Xian 3, WANG Guang-Ji 2*

1 Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing 210009, China
2 State Key Laboratory of Natural Medicines, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China;
3 School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China

Available online 20 Nov. 2013

[ABSTRACT] AIM: Silybin (SB), a major constituent of the milk thistle, has been used to treat several liver disorders. However, liver diseases were always accompanied by CYP450 dysfunction. This study was designed to explore the relationship between the hepatoprotective effect and CYP3A regulation of SB during thioacetamide (TAA)-induced rat liver injury. METHODS: Serum biochemical analysis and histopathological study were taken to evaluate the hepatoprotective effect of SB. α-SMA were detected by immunohistochemical analysis and cytokine release in rat liver was determined by ELISA assay. CYP3A and PXR expression were determined by RT-PCR and Western blot analysis, and CYP3A activity was based on the midazolam 4-hydroxylation reaction. Also, siRNA transfection was induced in HepG2 cells to evaluate the effect of PXR on cytotoxicity and CYP3A4 dysregulation caused by TAA. RESULTS: SB showed powerful hepatoprotective effects, and anti-inflammatory and anti-fibrosis effects, and reversed the loss of CYP3A and PXR in TAA-injured rat liver, and decreased PXR translocation into the cell nucleus. PXR silencing weakened the effect of SB on cytoprotection and CYP3A regulation. CONCLUSIONS: PXR was a very important factor of CYP3A regulation and might be the target of SB in TAA-induced liver disease. Also, because of the potential interactions of SB and co-administered medicines, it might be necessary to adjust the dosage in the clinical medication of liver disease.

[KEY WORDS] Silybin; Thioacetamide; Liver injury; Inflammation; CYP3A; PXR

[CLC Number] R965

[Article ID] 1672-3651(2013)06-0645-08

1 Introduction

*Silybum marianum* (L.) Gaertn. (Asteraceae), commonly known as milk thistle, has been used for decades as an herbal remedy, and also in an extracted formulation as a prescription drug for the treatment of a variety of liver disorders [1-3]. Silybin, a polyphenolic plant flavonolignan is the major active component of milk thistle, and is the active phytochemical responsible for the claimed benefits of silymarin extract to treat several liver disorders, such as hepatitis C virus infection [4] and non alcoholic steatohepatitis [5]. Silybin was reported to exert hepatoprotective effects as an antioxidant, and as an inhibitor of lipid peroxidation and free radical scavenger [6]. Silybin possesses anti-inflammatory properties on LPS-stimulated macrophages due to inhibition of inducible nitric-oxide synthase expression and tumor necrosis factor-alpha (TNF-α) production [7-8]. At the same time, silybin may exert effects on both phase I and phase II metabolic reactions. For instance, silybin has been shown to non-competitively inhibit denitronifedipine oxidation, mediated by CYP3A4 (Ki = 11 μmol·L−1), in human liver microsomes and competitive inhibition was reported for S(-)-warfarin 7-hydroxylation (CYP2C9; Ki = 19 μmol·L−1) [9-10]. Therefore, it is possible that silybin also has an impact on CYP450 in liver injury status, but there was little research about the *in vivo* action of SB on CYP450.

Thioacetamide (TAA) is well-known to induce both acute and chronic hepatic failure [11-13], and is widely applied to develop animal models of liver fibrosis and cirrhosis, because prolonged exposure to TAA always results in liver fibrosis and cirrhosis histologically similar to that in human viral hepatitis infections [14-16]. Thus, as a hepatotoxic and hepatocarcinogenic agent, TAA is a good choice as an inducer of
liver necrosis, cirrhosis, and carcinoma in animal model studies to mimic the human non-biliary liver diseases. Under TAA-induced hepatic injury status, the main liver P450 isoenzymes were dysregulated [17], especially CYP3A, one of the most important P450 enzymes in the liver. Because the CYP3A family genes produce key enzymes for the metabolism of more than 50% of prescription drugs, there has been a strong interest in understanding CYP3A gene regulation. Unfortunately, there was little investigation about CYP3A regulation of silybin a result of liver damage.

Pregnane X receptor (PXR) is a nuclear hormone receptor activated by a diverse array of endogenous hormones, dietary steroids, pharmaceutical agents, and xenobiotic compounds. It is expressed predominantly in the liver and intestine, both sites of steroid and xenobiotic metabolism. PXR activation directly stimulates the transcription of P450 enzymes in response to the presence of its ligands, and plays a central role in the transcriptional regulation of CYP3A4 and is related with inflammation [18-19]. In summary, it was hypothesized that the variation of drug metabolism enzymes as a result of liver injury was closely related with the status of liver damage and PXR inactivation, and that silybin could regulate CYP3A under TAA-induced liver injury through the PXR pathway. To test this hypothesis, both protein levels and enzyme activities of CYP3A and the variation of PXR were determined in chronic TAA-treated rat livers. Also, the hypothesis was confirmed in a TAA-induced HepG2 cell injury model. It was of interest to determine whether the loss of PXR was involved in irreversible enzyme inactivation, and the effect of silybin on this pathway. This study would help to understand the relationship between liver protection and drug metabolism enzymes, and further give a hint on the dosage regimen of co-administered silybin.

2 Materials and Methods

2.1 Materials

Thioacetamide (TAA) was obtained from Jiahui Medicine Chemical LLC. (Anhui, China). Dimethyl diphenyl dibcarboxylate was purchased from Hangzhou Dengyu Pharm & Tech Co., Ltd. pharmacy (Zhejiang, China). Silybin was purchased from Nanjing Qingze Pharmaceuticals Company (Jiangsu, China). Midazolam was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 4-Hydroxymidazolam, glucose 6-phosphate, NADP+, and glucose 6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore Corporation, Billerica, MA, USA). Phenylmethanesulfonyl fluoride and SDS-PAGE sample loading buffer were purchased from Becton Institute of Biotechnology (Jiangsu, China). Polyvinylidene difluoride membranes were obtained from Millipore (Shanghai, China). ELISA kit of rat IL-1β, IL-6, and IL-10 and TNF-α were from Excell Bio (Shanghai, China). Rabbit anti-rat CYP3A2 polyclonal antibody and rabbit anti-rat PXR polyclonal antibody were from Chemicon Corporation (USA). Mouse monoclonal antibody of α-SMA was from Abcam (UK). 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). Enhanced chemiluminescence kit was purchased from Pierce Chemical Company (Rockford, USA). siRNA designed according to PXR and the siRNA transfection reagent were from Invitrogen (USA). RNA extract reagent and SYBR Premix Ex Taq were from Takara Bio (Dalian, China). Nuclear and Cytoplasmic Extraction Reagent kit was from KeyGen (Nanjing, China).

2.2 Animals and experimental design

Male Sprague-Dawley rats (180–220 g) were obtained from the Academy of Military Medical Sciences, China. All rats were maintained in air-conditioned animal quarters 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 two weeks and then randomly divided into four groups with six animals in each group. TAA was intraperitoneally administered (200 mg·kg⁻¹, 3% in saline, twice a week for six weeks) to all groups of rats, except the normal control group which received normal saline injection instead. From the beginning of the seventh week, rats were intragastrically treated with CMC-Na suspension (groups I and II), SB (200 mg·kg⁻¹·d⁻¹, group III), or DDB (200 mg·kg⁻¹·d⁻¹, group V) for two weeks. At the end of the experimental treatments, blood samples were collected and then the rats were euthanized and the liver samples were immediately removed and frozen in liquid nitrogen.

2.3 Cell

The human Caucasian hepatocyte carcinoma (HepG2) cell line was purchased from the American Type Culture Collection (Bethesda, MD, USA) and was maintained in Dulbecco’s modified Eagle’s medium (Gibco BRL, Paisley, UK) supplemented with 10% fetal bovine serum (Gibco BRL, Paisley, UK) and 1% antibiotic-antimycotic (100 units·mL⁻¹ penicillin G sodium, 100 µg·mL⁻¹ streptomycin sulfate, and 0.25 µg·mL⁻¹ amphotericin B) in 5% CO₂ at 37 °C.

2.4 Serum biochemical analysis and histopathological study

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and total bilirubin (TBil) were determined by an automatic blood biochemical analyzer (Beckman Counter LX20, USA).

Slices of the same parts of the livers were cut off and fixed in formalin for at least 72 h, dehydrated in graded alcohol series, and embedded in paraffin. Sections of liver tissue (6 mm², 5 µm) were cut and stained with haematoxylin-eosin and examined for histopathological changes under the microscope (Olympus BH2, Japan). The images were taken using a Nikon Coolpix 990 camera at an original mag-
For immunohistochemical analysis, liver sections were fixed in 4% paraformaldehyde, paraffin-cut section, hydrated and then incubated in 3% hydrogen peroxide for 10 min. After three washes with PBS, the sections were placed in PBS supplemented with 3% bovine serum albumin for 30 min. Antibody of α-SMA was incubated overnight at 4 °C at a dilution of 1:200, a negative control was incubated with PBS instead of antibody. After washing with PBS, the sections were exposed to biotinylated universal secondary antibodies for 1 h, then to streptavidin biotin horseradish peroxidase solution. The reaction product was developed using 3,3′-diaminobenzidine tetrahydrochloride (Sigma). Sections were counterstained with hematoxylin for 20 s, dehydrated through graded alcohols, and mounted in resinous medium.

2.5 Determination of the release of cytokines

The amounts of IL-β, IL-6, and IL-10, and TNF-α in the rats liver samples were determined by specific ELISA kit (Excell Bio, Shanghai) following the instructions. Detection range: IL-β, 5–500 pg·mL⁻¹; IL-6, 5–500 pg·mL⁻¹; IL-10, 5–500 pg·mL⁻¹, and TNF-α, 5–500 pg·mL⁻¹.

2.6 Real-Time RT-PCR Analysis

Total RNA of HepG2 cells was isolated using RNA extraction reagent according to the manufacturer’s protocol. Quantitative real-time PCR assays were performed using SYBR Premix Ex Taq following the manufacturer’s instructions. PCR was performed in a volume of reaction mixture (10 μL) for each sample containing Universal Master Mix (1×), primers and probe (0.25 μmol·L⁻¹), and the RT product from 25 ng of RNA. After incubation at 50 °C for 2 min and 95 °C for 10 min, the PCR reaction was performed for 40 cycles: denaturation at 95 °C for 30 s, anneal at 60 °C for 30 s, and extension at 72 °C for 30 s. Primers for human CYP3A4 were: sense primer, 5′-CAATGGACTGCATAATAACCG-3′, and anti-sense primer, 5′-GAGCCAAATCTACCTCTTCACA-3′. Primers for the human housekeeping β-actin gene were: sense primer, 5′-GAGAAGGAGATCACAGCCT-3′, and anti-sense primer, 5′-GCTCATCCACATGCTGGA-3′, and β-actin was used to normalize gene expression in all the samples. All samples were run in triplicate simultaneously with negative controls. Melting curve analyses were performed in each real-time RT-PCR experiment. Pfaffl’s method [20] was applied for relative quantification of gene expression normalized to a housekeeping gene. The results are expressed as fold induction versus control vehicle-treated cells.

2.7 Nuclear protein extraction

Nuclear proteins from HepG2 cells were extracted using Nuclear and Cytoplasmic Extraction Reagent kit (KeyGen, Nanjing, China), according to the manufacturer’s instructions. A nuclear extract for each culture condition was obtained and stored at −80 °C until further analysis. Proteins were measured by the Lowry method [21].

2.8 Western blot analyses

Protein levels of CYP3A2 and PXR were determined by a Western blot analysis. Liver protein samples (10 μg of protein per lane) were separated by SDS polyacrylamide gel electrophoresis with an 8% polyacrylamide gel and transferred to a PVDF membrane by electroblotting. The PVDF sheets were blocked in 5% non-fat dry milk in TBST (0.05% Tween 20 in TBS) at 37 °C for one hour. The blots were incubated with primary antibodies diluted in 5% non-fat dry milk in TBST overnight at 4 °C, followed by incubation with goat anti-rabbit secondary antibodies for one hour. The primary antibodies against CYP3A2 and PXR were diluted 1:150 and 1:1000, respectively. For reference, β-actin was detected using a polyclonal antibody (1:200) for 1 hour, and then 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 Bioworld Technology Inc. USA. Proteins were detected by enhanced chemiluminescence on Kodak film (Sigma-Aldrich, St. Louis, MO, USA). Quantification of CYP3A2 and PXR levels was done by laser densitometry of the X-ray films. The density of the immunoreactive bands was analyzed using Image J software (NIH).
dissolved in 200 μL dimethylsulfoxide. Plates were analyzed in an automatic enzyme immunoassay instrument Powerwave 200 (Bio-Tek Instruments, Winooski, VT, USA) at 570 nm. Cell viability was defined relative to untreated control cells as follows: cell viability = absorbance of treated sample/absorbance of control.

2.12 Statistical analysis

The values of the experimental data were expressed as \( \bar{x} \pm s \). Statistical significances were evaluated using one-way ANOVA, followed by the post-hoc LSD method for multiple comparisons.

3 Results

3.1 TAA induced liver injury and hepatoprotective effects of SB

As shown in Table 1, TAA intoxication induced severe hepatic injury as evidenced by the tremendous enhancement of Tbil, ALT, AST, and ALP levels. SB treatment significantly lowered the serum Tbil, ALT, and AST levels, and became similar to the positive control DDB, validating the hepatoprotective effects of SB. Based on the haematoxylin–eosin stained tissue section analysis, TAA intoxication generated extensive changes in liver morphology, including inflammation, steatosis, fibrosis, and centrilobular necrosis. SB treatment largely reduced the injury caused by TAA, characterized by decreased necrotic zones and less steatosis (Fig. 1).

3.2 Anti-inflammatory effect of SB on TAA-induced liver injury in rats

To clarify the effect of SB on cytokine release, the hepatic levels of IL-1β, IL-6, IL-10, and TNF-α were determined by ELISA. As a result of TAA-induced rat liver injury, inflammation was promoted by high levels of pro-inflammatory interleukins, such as IL-1β and IL-6, and low level of IL-10. Also, there was a large release of TNF-α under TAA intoxication status. After two weeks administration of SB, the inflammations were strongly reversed with a significant decrease of IL-1β, IL-6, and TNF-α, and an increase of IL-10. This study confirmed the anti-inflammatory effect of SB on TAA-induced liver injury in rats (Fig. 3).

3.3 Regulation of SB on expression and activities of hepatic CYP3A2 in TAA-induced liver injury rats

In order to determine the effect of SB on hepatic CYP3A2, the levels of CYP3A2 and PXR in the livers were determined by Western blot analyses. Both hepatic

![Fig. 2 Immunohistochemistry analysis of α-SMA of rat livers and α-SMA positive staining was brown-reddish. (A) Normal, (B) TAA treated rats, (C) rats treated with SB 200 mg·kg⁻¹ + TAA, (D) rats treated with DDB 200 mg·kg⁻¹ + TAA](image)

![Fig. 1 Representative photomicrographs of histopathological studies of livers stained with haematoxylin and eosin (100 ×). (A) Normal, (B) TAA-treated rats, (C) rats treated with SB 200 mg·kg⁻¹ + TAA, (D) rats treated with DDB 200 mg·kg⁻¹ + TAA](image)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of SB on TAA-induced serum biochemical indicator variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Tbil (μmol·L⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>2.87 ± 0.60</td>
</tr>
<tr>
<td>TAA</td>
<td>3.47 ± 0.66</td>
</tr>
<tr>
<td>TAA + SB</td>
<td>2.22 ± 0.33</td>
</tr>
<tr>
<td>TAA + DDB</td>
<td>2.54 ± 0.33</td>
</tr>
</tbody>
</table>

* \( P<0.05 \), ** \( P<0.01 \) vs normal group; † \( P<0.05 \), †† \( P<0.01 \) vs TAA group
CYP3A2 and PXR protein levels significantly decreased in TAA-induced liver injury rats, however, they increased after SB treatment (Fig. 4A). Hepatic CYP3A2 activity was measured by the midazolam 4-hydroxylation reaction. Similar to the Western blot results, TAA-induced liver injury resulted in a significant loss of activity of CYP3A2 compared to the control group, and SB was capable of easing such loss (Fig. 4B).

Liver microsomal CYP3A2 activity was much lower than normal rats in the TAA-induced liver injury rats and recovered in SB-treated liver injury rats.

3.4 SB reversed the downregulation of TAA on PXR nuclear translocation

HepG2 cells were seeded in T-25 cell culture flasks and 24 h later SB (10, 50 μmol·L\(^{-1}\)) and 0.1% TAA were added. After 24 h, the cells were harvested to extract the cell nucleus. TAA treatment reduced the PXR translocation into the nucleus, while SB dose-dependently enhanced the PXR expression inside the cell nucleus, suggesting that SB probably regulated CYP3A4 transcription by promoting PXR translocation into the cell nucleus after TAA irritation (Fig. 5).

3.5 PXR plays an important role in CYP3A4 regulation in a TAA-induced HepG2 injury model

The transfection experiment was optimized to produce maximum silencing with minimal or no cytotoxicity. The optimal siRNA concentration was 50 nmol·L\(^{-1}\). The effect of siRNA transfection on PXR expression was investigated by Western blot analysis using protein extracted from the transfected HepG2 cells, and the silencing of PXR on HepG2 cells was confirmed as highly efficient (Fig. 6A). SB (2, 10, and 50 μmol·L\(^{-1}\)) dose-dependently reversed the down-regulation of the CYP3A4 mRNA level of TAA in the silenced control group. However, PXR silencing seemed to weaken the effect of TAA and SB, which implied the important role of PXR in CYP3A4 regulation after TAA intoxication (Fig. 6B). The MTT assay was used to determine the cytotoxicity of TAA, and the protective effect of SB, on HepG2 cells. SB (10 and 50 μmol·L\(^{-1}\)) was able to alleviate...
the cell loss caused by TAA treatment in the silenced control group (Fig. 7). Otherwise, PXR silencing influenced the action of both TAA and SB, which indicated that PXR was a very important factor in CYP3A4 regulation in the TAA-induced HepG2 injury model.

Fig. 6  PXR silencing on HepG2 cells and the effect of SB on CYP3A4 mRNA on TAA induced HepG2 cell injury. A. The efficient of silencing of PXR on HepG2 cells was validated by Western blot. PXR was barely expressed after siRNA transfection. B. SB was effective against the down-regulation of CYP3A4 mRNA by TAA, but this effect was weakened after PXR was silenced. C. The protective effect of SB on TAA-induced HepG2 cell injury was verified by the MTT test, but this protection almost vanished after PXR silencing. *P < 0.05, **P < 0.01 vs normal group; *P < 0.05, **P < 0.01 vs TAA group

4 Discussion

Silymarin, a flavonolignan from the milk thistle plant is used almost exclusively for hepatoprotection. Silymarin consists of four flavonolignan derivatives namely silybin, isosilybin, silydianin, and silychristin, among which, silybin is the most active and commonly used clinically. Silybin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts by anti-oxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory and liver regenerating mechanisms, and has clinical applications in alcoholic liver diseases and liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxin and drug-induced liver diseases and in diabetic patients [25-26]. Therefore, it is quite possible for SB to have drug-drug interactions in these various clinical applications.

However, there were only a few in vitro studies about the effect of silybin on drug metabolizing enzymes [18], and little is known about the in vivo effects. One study in humans demonstrated that silymarin administration decreased the excretion of bilirubin glucuronide in patients with liver cirrhosis due to alcohol abuse [27], although the clinical significance was not clearly indicated. Other reports on clinical hyperbilirubinemia or the interactions between silybin and drugs are lacking. Therefore, it is of importance to determine the influence of silybin on CYP450 enzymes in vivo, especially in liver disease. In this study, TAA was chosen as a hepatotoxic agent, since it is largely used as an inducer of liver necrosis, cirrhosis, and carcinoma in animal model studies. TAA diminishes the content of hepatic cytochrome P450 and inhibits the enzyme activity of the hepatic mixed function oxidase, and also binds to several subunits of glutathione-S-transferase (GST) and inhibits the expression of class a GST [28]. TAA-induced rat liver damage is an appropriate animal model to mimic human liver disease, especially the liver inflammation and fibrosis, which were established by cytokine release and α-SMA immunohistochemical analysis. SB showed powerful anti-inflammatory and anti-fibrosis effects by decreasing IL-1β, IL-6, and TNF-α levels, and α-SMA expression. It has long been known that inflammation and infection reduce the expression of hepatic cytochrome P450 genes which are involved in xenobiotic metabolism, such as CYP3A. TAA caused a significant down-regulation of CYP3A and PXR expression, which was significantly recovered by SB.

PXR, as the most important regulator of CYP3A, has been demonstrated to have a direct link between inflammation and drug metabolism by inhibiting the activity of NF-xB. Interestingly, NF-xB activation reciprocally inhibits PXR and its target genes, whereas inhibition of NF-xB enhances PXR activity [18-19]. This PXR–NF-xB axis provides a molecular explanation for the suppression of hepatic CYP3A mRNAs by inflammatory stimuli. In this study, it was found that TAA decreased the PXR expression inside the HepG2 nucleus which might be the reason for the down-regulation of CYP3A. SB was able to reverse the influence on PXR inside the cell nucleus caused by TAA. In the meantime, PXR silencing validated the effect of SB on hepatic cell protection and CYP3A regulation under TAA-induced cell damage.

In conclusion, PXR plays a crucial role in the regulation
of CYP3A and this role can explain the altered CYP450 regulation in liver disease conditions. Meanwhile, it is important to have a dosage regimen of co-administered silybin during liver disease treatment.

**Abbreviations**

SB, Silybin; TAA, thioacetamide; P450, cytochrome P450; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; Tbil, total bilirubin; fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PXR, pregnane X receptor; α-SMA, α-smooth muscle actin; TNF-α, tumor necrosis factor-alpha; IL-, interleukin; NADP⁺, β-nicotinamide adenine dinucleotide phosphate; LC-MS, liquid chromatography mass spectrometry

**References**


水飞蓟宾对硫代乙酰胺所致肝损伤状态下 CYP3A 的调节作用及机制

谢媛1, 郝海平2, 王洪2, 王兆先3, 王广基2*

1中国药科大学中药药理教研室, 南京 210009;
2中国药科大学天然药物活性组分与药效国家重点实验室, 药代动力学重点实验室, 南京 210009;
3中国药科大学药学院, 南京 210009

【摘要】目的: 水飞蓟宾(SB) 是植物奶蓟的主要成分, 长期以来被用于治疗各种肝脏疾病。由于肝病往往伴随有 CYP450 酶的功能失调, 因此本文以硫代乙酰胺(TAA) 引起的大鼠肝损伤为模型, 研究水飞蓟宾的保肝保护作用和对 CYP3A 的调控作用之间的关系。方法: 血清生化指标检测和肝脏病理切片实验评价水飞蓟宾的保肝作用。免疫组化实验测定 α-SMA 的表达, ELISA 试剂盒测定大鼠肝脏炎症因子的表达。实时定量 PCR 和 western blot 实验考察 CYP3A 和 PXR 的 mRNA 和蛋白表达水平变化。咪达唑仑 4-羟基化反应测定 CYP3A 的活性, siRNA 转染实验沉默 PXR 后考察 PXR 在硫代乙酰胺细胞毒和 CYP3A 调节中的作用。结果: 水飞蓟宾表现出明显的保肝、抗炎、抗纤维化作用, 并能有效逆转硫代乙酰胺导致的大鼠肝脏 CYP3A 和 PXR 表达减少以及 PXR 的入核减少。PXR 沉默实验显示 PXR 参与了水飞蓟宾的细胞保护和 CYP3A 调控过程。结论: PXR 是参与 CYP3A 调控的重要因子, 很可能是水飞蓟宾在硫代乙酰胺所致的肝损伤模型中的作用靶点, 同时也提示在治疗肝脏疾病时要注意与水飞蓟宾合用的药物可能与水飞蓟宾之间存在潜在的药物相互作用。

【关键词】水飞蓟宾; 硫代乙酰胺; 肝损伤; 炎症; CYP3A; PXR

【基金项目】国家自然科学基金(No. 81273586, 91029746, 81202590), 江苏省自然科学基金(No. SBK201240817), 中央高校基本科研业务费专项基金(No. JKQ2011040)资助