Swertiamarin Attenuates Experimental Rat Hepatic Fibrosis by Suppressing Angiotensin II–Angiotensin Type 1 Receptor–Extracellular Signal-Regulated Kinase Signaling

Shu Li, Qiglan Wang, Yanyan Tao, and Chenghai Liu

Institute of Liver Diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (S.L., Q.W., Y.T., C.L.); Baoshan Branch, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (S.L.); Shanghai Clinical Key Laboratory of Traditional Chinese Medicine (C.L.); E-Institute of TCM Internal Medicine, Shanghai Municipal Education Commission (C.L.), Shanghai, People’s Republic of China

Received April 3, 2016; accepted August 17, 2016

ABSTRACT

The rennin–angiotensin system (RAS) is crucial in hepatic fibrosis development, and therapies targeting this system may be a promising treatment for hepatic fibrosis. In this study, we investigated the effects of swertiamarin (Swe), an ethanol extract of Gentiana manshurica Kitag, on hepatic fibrosis and its underlying mechanisms through regulating RAS. Primary rat hepatic stellate cells (HSCs) were isolated and treated with angiotensin II (Ang II) with or without Swe and losartan. The proliferation and activation of HSCs were measured. Rat hepatic fibrosis was induced by intraperitoneal dimethylnitrosamine (DMN) injection for 4 weeks. Rats were treated with Swe or losartan from the third week until the end of the experiment. Hydroxyproline content in liver tissue was assayed with Jamall’s method, and liver collagen deposition was visualized using Sirius red staining. RAS components were analyzed by Western blot, immunoﬂuorescent staining, and real-time reverse-transcription polymerase chain reaction. The results showed that Swe signiﬁcantly inhibited Ang II-induced HSC proliferation and activation. Swe also signiﬁcantly suppressed DMN-induced α-smooth muscle actin production in rat livers and improved liver function. Swe partially inhibited Ang II-induced angiotensin type 1 receptor (AT1R) up-regulation and suppressed Ang II-induced extracellular signal-regulated kinase (ERK) and c-jun phosphorylation in HSCs. In the DMN-treated rats, Swe treatment signiﬁcantly inhibited the plasma Ang II levels. DMN-induced AT1R up-regulation, and phosphorylation of ERK and c-jun in rat liver were also inhibited by Swe. In conclusion, Swe may attenuate hepatic ﬁbrosis through inhibiting HSC activation by regulating the RAS.

INTRODUCTION

Hepatic ﬁbrosis, a dynamic process caused by many chronic liver diseases such as hepatitis B virus infection and alcohol abuse, can eventually lead to cirrhosis and even malignant tumors (Beljaars et al., 2002). Clinical and laboratory studies have indicated that ﬁbrosis is a reversible process, unlike the reversion of cirrhosis (Friedman and Bansal, 2006; Ismail and Pinzani, 2011; Kim et al., 2013; Czaja, 2014). Therefore, the development of therapies that could reverse ﬁbrosis could have a substantial clinical impact. Hepatic stellate cells (HSCs) are a central mediator of hepatic ﬁbrosis and play a crucial role in the process of hepatic ﬁbrosis (Hao et al., 2014; Tung et al., 2014). After HSC activation, extracellular matrix (ECM) production is increased, and the degradation of ECM is decreased, eventually leading to abnormal deposition of ECM and hepatic ﬁbrosis (Moreira, 2007; Das and Vasudevan, 2008). In view of the important role of HSCs in the production of ECM, HSCs are considered as a target in antifibrotic therapy (Safadi and Friedman, 2002).

The rennin–angiotensin system (RAS) is a master regulator of blood pressure and body ﬂuid homeostasis, as well as playing an important role in tissue remodeling (Ruiz-Ortega et al., 2001b). In recent years, RAS was observed to be involved in the process of hepatic ﬁbrosis. Angiotensin II (Ang II), the main effector hormone of RAS, regulates key steps in the tissue ﬁbrosis process through the angiotensin type I receptor (AT1R) (Mezzano et al., 2001). In the carbon tetrachloride (CCL4)-induced rat hepatic ﬁbrosis model, the mRNA expression of angiotensinogen increases at the early stage of hepatic ﬁbrosis (Lu et al., 2011). In patients with cirrhosis, systemic Ang II is frequently up-regulated (Vilas-Boas et al., 2009).
Increased systemic Ang II induces contraction and proliferation of human HSCs, exacerbates hepatic fibrosis, and promotes inflammation, oxidative stress, and vascular damage (Bataller et al., 2000; Bataller et al., 2005).

Interestingly, activated HSCs also synthesize Ang II and express components of RAS, including AT1R (Bataller et al., 2003b). Accordingly, inhibiting the generation of Ang II and/or blocking the binding of Ang II to AT1R could markedly attenuate hepatic fibrosis (Jonsson et al., 2001; Ohishi et al., 2001; Paizis et al., 2001). Therefore, therapies targeting RAS may represent a promising treatment of hepatic fibrosis in the setting of chronic liver disease.

In our institute, we have focused on developing effective therapeutic methods for the treatment of hepatic fibrosis for many years. Gentiana manshurica Kitag, an herb belonging to the Gentianaceae family, is used in traditional Chinese medicine to treat many liver diseases. Gentiana manshurica Kitag can reverse acute alcohol-induced liver steatosis (Lian et al., 2010) and prevent acetaminophen-induced acute hepatic injury in mice by inhibiting c-Jun N-terminal kinase (JNK)/extracellular signal-regulated kinase (ERK) pathways (Wang et al., 2010a). Swertiamarin (Swe), the ethanol extract of Gentiana manshurica Kitag, exerts antioxidant and hepatoprotective effect against β-galactosamine-induced liver damage in rats (Jaishree and Badami, 2010). In our preliminary experiments, Swe also showed a significant effect on the proliferation and activation of HSCs induced by Ang II. Therefore, in the present study, we further investigated the antihepatic-fibrosis effect of Swe. In view of the importance of RAS in liver fibrogenesis, we determined whether the mechanism of Swe action occurred by regulation of RAS.

Materials and Methods

Chemicals and Drugs. Swe was obtained from Winherb Medical Technology (Shanghai, People’s Republic of China). The purity of Swe was 98%, molecular weight 374.34, and molecular formula C16H22O10. Swe was dissolved in dimethylsulfoxide at a concentration of 20 mM and stored at −20°C for future use. The 5-ethyl-2′-deoxyuridine (EdU) cell proliferation kit (Cell-Light EdU DNA Cell Proliferation Kit) was purchased from RiboBio (Guangzhou, People’s Republic of China). Ang II was purchased from Sigma-Aldrich (St. Louis, MO). Antibodies against angiotensin type 1 (AT1, sc-1173; Santa Cruz Biotechnology, Dallas, TX), phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2; Santa Cruz Biotechnology), total ERK2 (Santa Cruz), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Santa Cruz Biotechnology), α-smooth muscle actin (α-SMA; Abcam, Cambridge, MA), GAPDH (KangChen Bio-tech, Shanghai, People’s Republic of China), β-tubulin (Cell Signaling Technology, Beverly, MA), c-Jun (Cell Signaling Technology), and p-c-Jun (Cell Signaling Technology) were used in this study.

HSC Isolation and Culture. Primary HSCs were isolated from normal rat livers by perfusion with pronase, followed collagenase and Nycodenz density-gradient centrifugation (Friedman et al., 1992). Primary HSCs were cultured with Dulbecco’s modified Eagle’s medium supplemented with 20% fetal bovine serum. Primary HSCs were seeded in 6-, 12-, and 96-well plates on day 3. The experiments were performed in accordance with the Helsinki Declaration of 1975.

Lactate Dehydrogenase Assay. HSCs were seeded at a density of 5,000 cells per well in 96-well plates. After the cells had grown for 24 hours under normal growth condition, the medium was replaced with DMEM containing different concentrations of swertiamarin. The cells were incubated for another 24 hours. The activity of lactate dehydrogenase (LDH) in cell-culture medium (extracellular LDH) or intracellular LDH was determined using a commercial LDH kit according to the manufacturer’s instructions. The amount of LDH released into the extracellular medium was expressed as the percentage of the total intracellular and extracellular content.

Cell Proliferation Analysis. To assess cell proliferation, HSCs were plated on 96-well plates. Upon reaching 60% confluence, cells were treated with 0.1 μM Ang II, different concentrations of Swe, or Losartan for 24 hours. The cells were incubated under standard conditions in complete medium. Cell proliferation was detected using the incorporation of EdU from the EdU Cell Proliferation Assay Kit. Briefly, the cells were incubated with 50 μM EdU for 3 hours before fixation, permeabilization, and EdU staining, which were performed according to the manufacturer’s protocol. The cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) at a concentration of 1 μg/ml for 10 minutes. EdU-positive and EdU-negative cells were determined by fluorescent imaging. Images were taken using Cellomics ArrayScan VTI HCS Reader and analyzed using Cellomics Cell Health Profiling BioApplication software (Thermo Fisher Scientific, Waltham, MA).

Immunofluorescence. Cells were plated in 96-well plates. After growing for 24 hours under normal growth conditions, the medium was replaced with DMEM containing Ang II (0.1 μM) (Oesterreich et al., 2009) and different concentrations of Swe. The cells were incubated for another 24 hours. Cells were then washed twice with cold phosphate-buffered saline and fixed in 4% paraformaldehyde for 10 minutes.

Immunofluorescence was performed to detect the F-actin and α-SMA, as described previously (Sohail et al., 2009). Images were taken using Cellomics ArrayScan VTI HCS Reader and analyzed using Cellomics Cell Health Profiling BioApplication software (Thermo Fisher Scientific).

Animals. Male Wistar rats (6–8 weeks old) were obtained from the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences, and were maintained in a room under temperature control at 23 ± 2°C and a 12-hour light/dark cycle. The protocol was approved by the Committee on the Ethics of Animal Experiments of Shanghai University of Traditional Chinese Medicine, People’s Republic of China. All animals received humane care during the study with unlimited access to chow and water.

The rats were randomly divided into five treatment groups: control (n = 15), dimethylnitrosamine (DMN) (n = 15), Swe low-dose (Swe 15 mg/kg) (n = 15), Swe high-dose (Swe 20 mg/kg) (n = 15), and losartan (n = 15). To induce liver fibrosis, 10 μg/kg DMN was given by intraperitoneal injection 3 consecutive days per week over a period of 4 weeks by the Ala-Kokko et al. (1987) procedure with minor modifications (Pines et al., 1997; Su et al., 2013). From the third week of DMN injection, rats in the Swe 15 mg/kg, Swe 20 mg/kg, and losartan groups were treated with Swe at a dose of 15 mg/kg per day, 20 mg/kg per day, and losartan at a dose of 10 mg/kg per day, respectively. Rats in the control and DMN groups were treated with equal amount of vehicle.

After 2 weeks of treatment, rats were anesthetized with 2% pentobarbital sodium, and the blood samples were obtained from the vena cava inferior. A portion of each liver was fixed in 10% phosphate-buffered formalin for histologic studies after paraffin embedding. The remainder was snap-frozen in liquid nitrogen and stored at −80°C for Western blot analysis.

H&E and Sirius Red Staining. The left lateral lobe of the liver was sliced, and the tissue slices were fixed in 10% buffered-neutral formalin for 24 hours. The fixed liver tissue slices were embedded in paraffin, sectioned, deparaffinized, and rehydrated using standard techniques. Sections (5 μm) were subjected to H&E and Sirius red staining as described elsewhere (Wang et al., 2010b). An arbitrary scope was given to each microscopic field viewed at an original magnification of 200×.

Hydroxyproline Content Measurement. Hepatic hydroxyproline (Hyp) content was measured with HCl hydrolysis according to Jamall’s method. Briefly, 100 mg of all liver samples were
homogenized and hydrolyzed in 6 M HCl at 110°C for 18 hours. Hydrolysates were filtrated with 3-mm filter paper and dried at 40°C. The samples were then incubated with Ehrlich’s solution (25% [w/v] p-dimethylaminobenzaldehyde and 27.3% [v/v] perchloric acid in isopropanol) at 50°C for 90 minutes and measured at A_558 nm. All results were normalized by total protein concentration and calculated using a standard curve.

**Serum Biochemical Measurements.** Serum alanine aminotransferase, aspartate aminotransferase, albumin, and total bilirubin levels were measured according to the manual’s instructions (Nanjing JianCheng Bioengineering Institute, People’s Republic of China).

**Measurement of Plasma Ang II Levels.** Blood was collected from the inferior vena cava into a chilled glass tube containing protease inhibitors and Enalapril to prevent ex vivo conversion of Ang I to Ang II. Samples were eluted from the column with 90% methanol, and then dried and reconstituted for the radioimmunoassay. The radioimmunoassay for Ang II was performed using 125I-angiotensin II (Perkin-Elmer, Foster City, CA) and rabbit anti-Ang II antibody (Phoenix Pharmaceuticals, Belmont, CA) with cross-reactivity of <2% for Ang II precursors and its degradation products. After incubation for 2 days at 4°C, bound and free Ang II were separated using dextran-coated charcoal. The supernatant was washed in phosphate-buffered saline/Tween, the blots were incubated with fluorescence-coupled secondary antibody. The signals were visualized using the Odyssey Imaging System (Li-Cor Biosciences).

**Real-Time Reverse-Transcription Polymerase Chain Reaction.** Total RNA was isolated using the TRIzol Reagent (Invitrogen, Shanghai, People’s Republic of China), according to the manufacturer’s protocol. The RNA concentration was determined using a NanoDrop Spectrophotometer. We generated cDNA using 1 µg of total RNA in a final reaction volume of 20 µl by use of the first-strand cDNA synthesis kit (Toyobo, Osaka, Japan), according to the manufacturer’s protocol. Quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) was performed with an ABI ViiA7 Real-time PCR system. The primers, along with their sequences, are listed in Table 1. The PCR mixtures contained 2 µl of cDNA, 10 µl of SYBR Premix 2×, and 0.25 µM of forward and reverse primers, for a total volume of 20 µl. Reactions were started with a polymerase activation step at 94°C for 10 minutes; followed by 40 cycles of 94°C for 10 seconds, 60°C for 20 seconds, and 72°C for 25 seconds. Fluorescence data were acquired after each cycle. The absence of nonspecific products was verified after each run by melting curve analysis. The relative gene quantities were calculated by the 2^(-DDCT) method in comparison with the expression levels of GAPDH.

**Statistical Analysis.** Data are expressed as mean ± S.D. Data were analyzed using one-way analysis of variance as well as the least significant difference test. *P < 0.05* was considered statistically significant.

---

**TABLE 1** The primers used in this study for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences</th>
<th>Gene Bank Accession No</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>5′-AAGCAGTGCCGACAGGAACTCA-3′</td>
<td>AY550625.1</td>
<td>77</td>
</tr>
<tr>
<td>α-SMA</td>
<td>Forward 5′-ACCACGGGGAATGACGCTT-3′</td>
<td>NM_031004.2</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>Reverse 5′-CTGTCAGCAATGGCCTGGTA-3′</td>
<td>NM_031144.3</td>
<td>211</td>
</tr>
</tbody>
</table>

---

**Fig. 1.** Swe inhibited the proliferation of HSCs. (A) The amount of LDH released into the extracellular medium was expressed as the percentage of the total intra- and extracellular content compared with the control group. HSCs were treated with angiotensin II (Ang II, 0.1 µM) with or without Swe (2.4, 6, 15 µM) and losartan (1 µM) for 24 hours. The proliferation of HSCs was determined using a commercial EdU cell proliferation kit. (B) The typical images of EdU staining (original magnification, 200×; red: 4,6-diamidino-2-phenylindole (DAPI); blue: EdU). (C) Quantitative analysis of the fluorescence intensity of EdU staining. Each bar represents the mean ± S.D. (*n = 6*). *P < 0.05, **P < 0.01, compared with the control group; ***P < 0.01, compared with the Ang II group.
Results

Swe Inhibited HSC Proliferation. Primary HSCs were incubated with 5 to 100 μM of for 24 hours and then assayed using the LDH method (Fig. 1A). The results showed that 15 μM Swe had no toxic effect on HSCs. HSC proliferation was determined with the EdU cell proliferation assay (Pipparelli et al., 2013). After 24 hours of incubation, Ang II significantly promoted the proliferation of HSCs (by 60%) when compared with control (Fig. 1, B and C). To determine the effect of Swe on HSCs proliferation, cells were cotreated with Ang II and Swe (2.4 μM, 6 μM, or 15 μM). Swe significantly suppressed Ang II-induced HSC proliferation at 6 and 15 μM. However, 2.4 μM of Swe showed little effect on HSC proliferation. Losartan also significantly suppressed the proliferation of HSCs (Fig. 1, B and C).

Swe Inhibited the Activation of HSCs. To evaluate the effect of Swe on Ang II-induced HSC activation, we examined the expression of α-SMA. As shown in Fig. 2, A–D, immunofluorescence staining and Western blot showed that α-SMA expression was significantly enhanced by Ang II and was reversed by losartan and Swe treatments in a concentration-dependent manner (Fig. 2, A–D). Real-time RT-PCR also showed that the Ang II-induced up-regulation of α-SMA mRNA was significantly suppressed by 6 and 15 μM of Swe and 1 μM of losartan (Fig. 2E).

The actin cytoskeleton plays an important role in defining cell shape and morphology. In this study, we also examined F-actin reorganization during the conversion from quiescent HSCs to myofibroblasts after Ang II administration. The results revealed that Ang II induced significant F-actin reorganization (Fig. 2, F and G), forming abundant long stress

![Figure 2](image-url)

Fig. 2. Swe inhibited the activation of HSCs. HSCs were treated with Ang II (0.1 μM) with or without Swe (2.4, 6, 15 μM) and losartan (1 μM) for 24 hours. The expression of α-SMA was determined by (A) immunofluorescence staining, (B) Western blot, and (E) real-time RT-PCR. (F) The expression of F-actin was examined by immunofluorescence staining. (C, D, and G) Quantitative data of α-SMA and F-actin expression, respectively. Each bar represents the mean ± S.D. (n = 3). *P < 0.01, compared with the control group; *P < 0.05, **P < 0.01, compared with the Ang II group.
fibers in HSCs. Different concentrations of Swe blocked these processes.

**Swe Regulated RAS-Related Molecules.** To explore the mechanisms of the inhibition of Ang II-induced HSC activation by Swe, we tested the expression of RAS-related molecules by Western blot and real-time RT-PCR. Ang II treatment significantly up-regulated the expression of AT1R (Fig. 3, A and B). Losartan and Swe treatment partly reversed Ang II-induced AT1R up-regulation. Real-time RT-PCR showed that Ang II treatment significantly increased the transforming growth factor β1 (TGF-β1) mRNA level (Fig. 3C). Both losartan and Swe suppressed the up-regulation of TGF-β1 mRNA induced by Ang II (Fig. 3C).

The activation of ERK and activator protein-1 (AP-1) is associated with cell growth. Therefore, we next examined the effect of Swe on the phosphorylation of ERK and AP-1. Ang II treatment induced a rapid phosphorylation of ERK1/2 (Fig. 3, D and E). Treatment of Ang II for 3 hours also induced phosphorylation of c-jun, a subunit of AP-1. Swe significantly suppressed Ang II-induced phosphorylation of ERK1/2 and c-jun, in a dose-dependent manner (Fig. 3, D and E). Furthermore, the nuclear translocation of c-jun was also inhibited by Swe (Fig. 3F).

**Swe Ameliorated Liver Fibrosis Induced by DMN in Rats.** The activation of HSCs plays a vital role in the progress of hepatic fibrosis, and our results suggested that Swe might be an effective agent in the treatment of hepatic fibrosis. Therefore, we further investigated the effect of Swe on DMN-induced rat hepatic fibrosis.

The excessive and disorganized deposition of collagens is a major pathogenic feature of fibrotic diseases. As shown in Fig. 4, A and B, DMN treatment induced a significant increase of
collagen expression, as determined by Sirius red staining. Treatment with Swe for 2 weeks resulted in an alleviation of collagen deposition (Fig. 4, A and B). Losartan also attenuated DMN-induced collagen overexpression in rat livers. Western blot analysis showed that α-SMA expression in DMN-treated rats was significantly increased and was then inhibited by Swe and Losartan (Fig. 4, C and D).

In DMN-treated rats, the hydroxyproline (Hyp) content was also significantly elevated compared with that of the control (Fig. 4E). Treatment with Swe or losartan improved the Hyp content significantly (Fig. 4E).

**Swe Improved DMN-Induced Rat Hepatic Injury.** DMN significantly decreased the body weight and liver/body ratio of rats compared with the control (Fig. 5, A and B). The rat body weight and liver/body ratio in the Swe and losartan groups were significantly increased compared with those in the DMN group (Fig. 5, A and B).

As shown by H&E staining, the liver showed normal cellular architecture in the control group (Fig. 5C). However, DMN induced severe histopathologic changes in the rat liver, such as centrilobular hepatic necrosis and infiltrating lymphocytes. Treatment with Swe and losartan partly prevented the histopathologic changes associated with DMN-induced hepatotoxicity (Fig. 5C). DMN treatment also significantly decreased the albumin level and increased the alanine aminotransferase, aspartate aminotransferase, and total bilirubin levels in rat serum (Fig. 5, D–G). Swe and losartan treatment significantly improved DMN-induced liver function injury.

**Swe Regulated the RAS Signaling Molecules In Vivo.** To further confirm whether Swe could also regulate RAS in vivo, we determined the levels of RAS-associated molecules in rats. In the DMN-induced rat hepatic fibrosis model, the expression of AT1R was significantly up-regulated (Fig. 6, A and B). Losartan, a blocker of AT1R, significantly decreased that of AT1R compared with the DMN group. Swe treatment also suppressed DMN-induced abnormal expression of AT1R. Phosphorylated ERK and c-jun were also significantly elevated in DMN-alone treated rats (Fig. 6, A, C, and D). Cotreatment with Swe or Losartan inhibited DMN-induced ERK and c-jun phosphorylation.

In the DMN group, the Ang II level in rat serum was significantly increased compared with control group (Fig. 6E). In the Swe high-dose and low-dose groups, Ang II levels were significantly down-regulated compared with the DMN group (Fig. 6E). However, the Ang II level of the losartan group was higher than that of the DMN group (Fig. 6E).

**Discussion**

Hepatic fibrosis, a reversible wound-healing response, is characterized by abnormal ECM deposition. It is a multifunctional process that involves HSC, cytokines, chemokines, and
various growth factors and results from a disruption of homeostatic mechanisms that maintain the liver ecosystem (Friedman, 2008; Malhi and Gores, 2008). HSCs, called lipid storage cells, lipocytes, or Ito’s cells, are perisinusoidal cells that normally reside in the space of Disse and contain numerous retinoid and lipid droplets (Geerts, 2001; Iredale, 2007).

In response to injury, quiescent HSCs, involved in the depletion of vitamin A storage and lowering of retinol chain expression, acquire contractility and activate into collagen type I, a-SMA myofibroblasts. HSC is the main cell type responsible for excessive deposition of connective tissue components in response to liver injury. The activation of HSC is the central event in the development of hepatic fibrosis. Therefore, many effective therapeutic approaches focus on regulating HSC activation and proliferation.

In the process of hepatic fibrosis, the components of RAS have attracted increasing attention (Zhang et al., 2013). RAS accelerates inflammation, tissue repair, and fibrogenesis through Ang II and its receptor AT1R (Kisseleva and Brenner, 2011). Prolonged administration of Ang II directly activates HSCs and causes hepatic fibrosis in rodents (Bataller et al., 2005; Zhang et al., 2014), while AT1R knockout may protect mice from hepatic fibrosis (Kanno et al., 2003; Yang et al., 2005). In the present study, Swe inhibited Ang II- and DMN-induced AT1R up-regulation in HSCs and rat liver, respectively. In addition, Swe down-regulated plasma Ang II levels in DMN-treated rats. These results indicate that the down-regulation of Ang II–AT1R signaling is involved in the antihepatic fibrosis effect of Swe.

Thus, the in vitro results indicate that Swe may be an effective agent for treating hepatic fibrosis. Therefore, we studied the effects of Swe on hepatic fibrosis in vivo. In the DMN-induced rat hepatic fibrosis model, Swe significantly inhibited the expression of a-SMA and collagen, and improved the rat liver function, exhibiting a good therapeutic effect. These data suggest that Swe is a good candidate for the treatment of hepatic fibrosis.

The profibrotic activity of Ang II is mainly mediated by AT1R (Wang et al., 2005; Yang et al., 2005; Li et al., 2007). Consistent with previous studies (Lu et al., 2011; Dasuri et al., 2013; Shahid et al., 2013), we also demonstrated that the AT1R level was up-regulated in Ang II-treated HSCs and the livers of DMN-treated rats (Li et al., 2012). In the present study, Swe inhibited Ang II- and DMN-induced AT1R up-regulation in HSCs and rat liver, respectively. In addition, Swe down-regulated plasma Ang II levels in DMN-treated rats. These results indicate that the down-regulation of Ang II–AT1R signaling is involved in the antihepatic fibrosis effect of Swe.
many cell types, such as human endothelial cells, vascular smooth muscle cells, and rat aortic cells (Zhu et al., 2002; Su et al., 2006). Our study also showed that Ang II stimulation produced a fast phosphorylation of ERK1/2 and c-jun. Swe treatment significantly inhibited Ang II-induced and DMN-induced ERK and c-jun phosphorylation in HSCs and rat livers, respectively. These results suggest that Swe may exert antihepatic fibrosis effects by suppressing Ang II–AT1R signaling and consequently suppressing ERK and c-jun phosphorylation.

Although we showed that Swe ameliorated DMN-induced hepatic fibrosis and inhibited the Ang II and AT1R in rats, the direct correlation between the antihepatic fibrosis effects of Swe and RAS remains unclear. In addition to RAS, many signaling pathways are involved in hepatic fibrosis induced by DMN or other chemicals; nevertheless, our Ang II-induced hepatic fibrosis model appears to be the most useful for verifying the direct action of Swe on RAS.

In conclusion, our study demonstrated that Swe is an effective agent to treat experimental hepatic fibrosis. Swe inhibited Ang II-induced HSC activation and proliferation. Swe also attenuated DMN-induced rat hepatic fibrosis and improved liver function. The mechanisms involved in Swe’s action include down-regulating Ang II and AT1R expression and repressing ERK and c-jun activation. Our results indicate that Swe is a potentially effective agent for treating hepatic fibrosis through the regulation of RAS.

References


Address correspondence to: Dr. Yanyan Tao, Institute of Liver Diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Pudong New Area, Shanghai 201203, People’s Republic of China. E-mail: yanyantao@126.com or Dr. Chenghai Liu, Institute of Liver Diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Pudong New Area, Shanghai 201203, People’s Republic of China. E-mail: chenghaliufe@hotmail.com