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Schisandrin A and B induce organic anion transporting polypeptide 1B1 transporter activity

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Organic anion transporting polypeptide 1B1 (OATP1B1) is the most important transporter in the organic anion transporting polypeptide family. OATP1B1 plays an important role in the hepatic uptake of many endogenous compounds and xenobiotics, including many clinical drugs. At present, the combinational usage of Chinese traditional herbal medicines and conventional chemical pharmaceuticals may affect the activity of enzymes and transporters activity and cause absorption of their substrates and metabolic changes. In this study, we aimed to investigate the effect of schisandrin A, schisandrin B and tanshinone IIA, which were extracted from medicinal plants, on OATP1B1 activity. HepG2 cells are used as *in vitro* models for OATP1B1 activity studies. A combination of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tertazolium bromide (MTT) assays, real-time RT-PCR, and transporter activity analysis were employed. We found that schisandrin A and B increased OATP1B1 mRNA levels by 1.81-fold ($p < 0.01$) and 1.87-fold ($p < 0.01$) at concentration of 10 μM, respectively. Schisandrin A of 1 μM and 10 μM and schisandrin B of 10 μM significantly increased the uptake of [^{3}H] estrone-3-sulfate ($p < 0.05$ or $p < 0.01$). Tanshinone IIA had no effect on the mRNA expression and transport activity of OATP1B1 at nontoxic concentrations. Our study suggests that schisandrin A and B induced OATP1B1 expression and increased its transporter activity in HepG2 cells.

1. Introduction

OATP1B1, encoded by the *SLCO1B1* gene, is primarily expressed in the basolateral (or sinusoidal) membrane of hepatocytes in the liver. It mediates the uptake of substrates from blood into the liver (Abe et al. 1999). OATP1B1 is responsible not only for the biotransformation of some endogenous compounds, such as bile acids, bilirubin, and thyroid hormones, but also for the metabolism of many important drugs such as antineoplastic, antihyperlipidemic, antihypertensive, hypoglycemic, HIV protease inhibitors, and antibacterial compounds (Abe et al. 1999; Cui et al. 2001; Niemi et al. 2011). OATP1B1 plays a critical role in drug metabolism and drug-drug interaction in humans.

The use of Chinese traditional herbal supplements is more and more popular around the globe due to its safety and efficiency. The herbal medicines are often administered in combination with conventional chemical pharmaceuticals to improve the therapeutic efficacy and to reduce adverse effects. However, some herbal medicines may inhibit, or induce cytochrome P450 (CYP450) enzymes and transporter activity, causing undesired herb–drug interactions.

Wu Wei Zi (*Schisandra chinensis* (Turcz.) Baill.) and Danshen (*Radix Salvia miltiorrhiza*) have been widely used in China and Japan. Wu Wei Zi is primarily used to treat chronic hepatitis and liver dysfunction. The major clinical indication of Danshen is coronary heart disease and cerebrovascular disease. Previous studies revealed that both of them could change CYP3A4 activity and then alter the pharmacokinetics of co-administered CYP3A4 substrates (Yu et al. 2009; Qin et al. 2010). Wu Wei Zi and Danshen are often co-administered with substrates of OATP1B1. So far, it is not known yet whether Wu Wei Zi and Danshen can regulate OATP1B1 expression, resulting in a change of OATP1B1 activity.

This study aimed to investigate the effect of schisandrin A and B, extracted from Wu Wei Zi, and tanshinone IIA, extracted from Danshen, on the regulation of OATP1B1 expression.

2. Investigations, results and discussion

2.1. Effects of schisandrin A, B and tanshinone IIA on the viability of HepG2 cells

We studied the viability of cells treated with different concentrations of tanshinone IIA and schisandrin A, B using the MTT assay. Schisandrin A or B showed no significant effect on cell viability at different concentrations of 0.1, 1, 10, 100, and 200 μM (Fig. 1). Tanshinone IIA had a dose-dependent inhibition on cell growth (Fig. 1). When Tanshinone IIA was applied at concentrations of > 10 μM, cell viability was significantly decreased ($p < 0.05$).

2.2. Schisandrin A and B induce the expression of OATP1B1 mRNA

To find out whether tanshinone IIA and schisandrin A, B could induce the expression of OATP1B1 mRNA in HepG2 cells, real

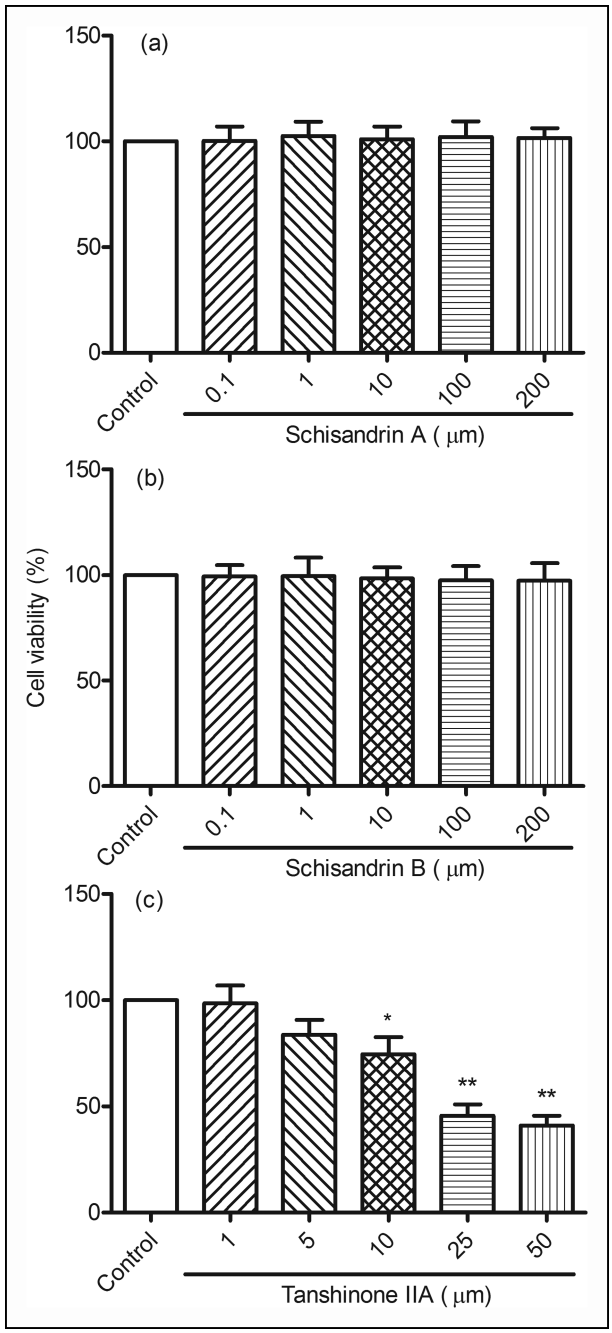

Fig. 1: Effect of schisandrin A, schisandrin B and tanshinone IIA on the viability of HepG2 cell. (a): schisandrin A. (b): schisandrin B. (c): tanshinone IIA. Data are presented as the mean ± SD. Compared with the control group $*P<0.05$ and $**P<0.01$.

time PCR assays were performed. The 48-h exposure to schisandrin A and B caused significant induction of OATP1B1 mRNA expression levels in HepG2 cells (Fig. 2). At a concentration of 10 μM, schisandrin A and B induced a 1.81-fold and 1.87-fold increase in the levels of OATP1B1 mRNA compared with the DMSO control, respectively. But tanshinone IIA had no effect on the levels of OATP1B1 mRNA expression at concentrations of 5 μM.

2.3. Induction of OATP1B1 mediated [^{3}H] estrone-3-sulfate uptake by schisandrin A and B

After pretreating the cells with schisandrin A and B at different concentrations (0.1, 1, 10 μM) for 48 h, we found that [^{3}H] estrone-3-sulfate uptake was significantly increased in

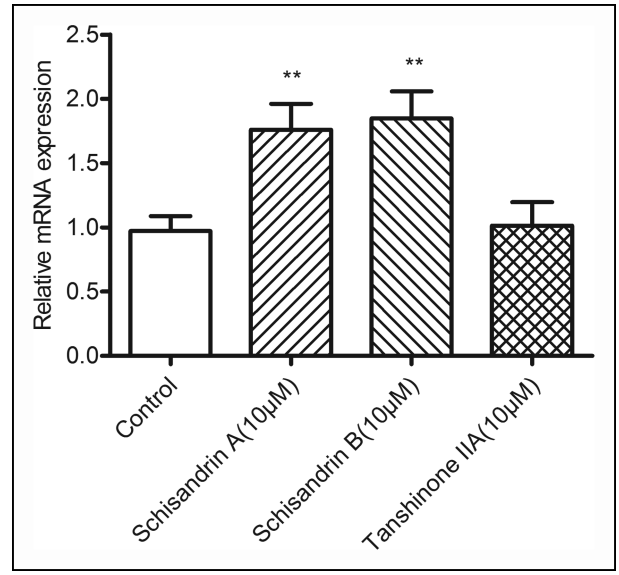

Fig. 2: Effect of schisandrin A and B on OATP1B1 mRNA expression in HepG2 cells. Data are presented as the mean ± SD. Compared with the control group $**P<0.01$.

HepG2 cells ($p<0.05$ or 0.01). Tanshinone IIA had no effect on OATP1B1 transport activity at concentrations of (0.1, 1, 5 μM) (Fig. 3).

Schisandrin A at concentrations of 0.1 μM did apparently not enhance in [^{3}H] estrone-3-sulfate uptake over the DMSO control. However, at concentrations of 1 μM and 10 μM, schisandrin A significantly induced [^{3}H] estrone-3-sulfate uptake over the DMSO control ($p<0.05$).

Schisandrin B, at concentrations of 0.1 μM and 1 μM, did not cause any changes in uptake of [^{3}H] estrone-3-sulfate in HepG2 cells. But, at concentrations of 10 μM, schisandrin B dramatically increased [^{3}H] estrone-3-sulfate uptake compared to the DMSO control ($p<0.01$).

3. Discussion

The present study showed for the first time, that, schisandrin A and B increased OATP1B1 mRNA expression and transporter activity in HepG2 cells, which exhibit low but sufficiently detectable OATP1B1 mRNA expression (Libra et al. 2006; Rodrigues et al. 2009).

At a concentration of 10 μM, schisandrin A and B significantly induced OATP1B1 mRNA levels. Schisandrin A at 1 μM and 10 μM and schisandrin B at 10 μM significantly significantly increased [3H] estrone-3-sulfate uptake. But, tanshinone IIA had no effect on the levels of OATP1B1 mRNA expression and OATP1B1 transport activity at nontoxic concentrations ($\leq$ 5 μM).

Schisandra is one of the traditional Chinese medicines, which are used widely. It has many active ingredients and pharmacologic functions. In previous studies, Schisandra in A and gomisin A extracted from *Schisandra*, could inhibit cytochrome P450 3A4 activity with IC50 values of 32.02 μM and 1.39 μM, respectively. They also reversed P-glycoprotein-mediated multidrug resistance by P-gp substrate interaction (Wan et al. 2006; Fong et al. 2007; Wan et al. 2010). The components of *Schisandra* can change the activity of metabolic enzymes and transporters, but different components of Schisandra have different effects.

It is well known that OATP1B1 is strongly associated with statin-induced myopathy. A common genetic variant of OATP1B1 (*SLCO1B1* c.521T > C) markedly reduces the hepatic uptake of statins, which results in elevated blood concentration and

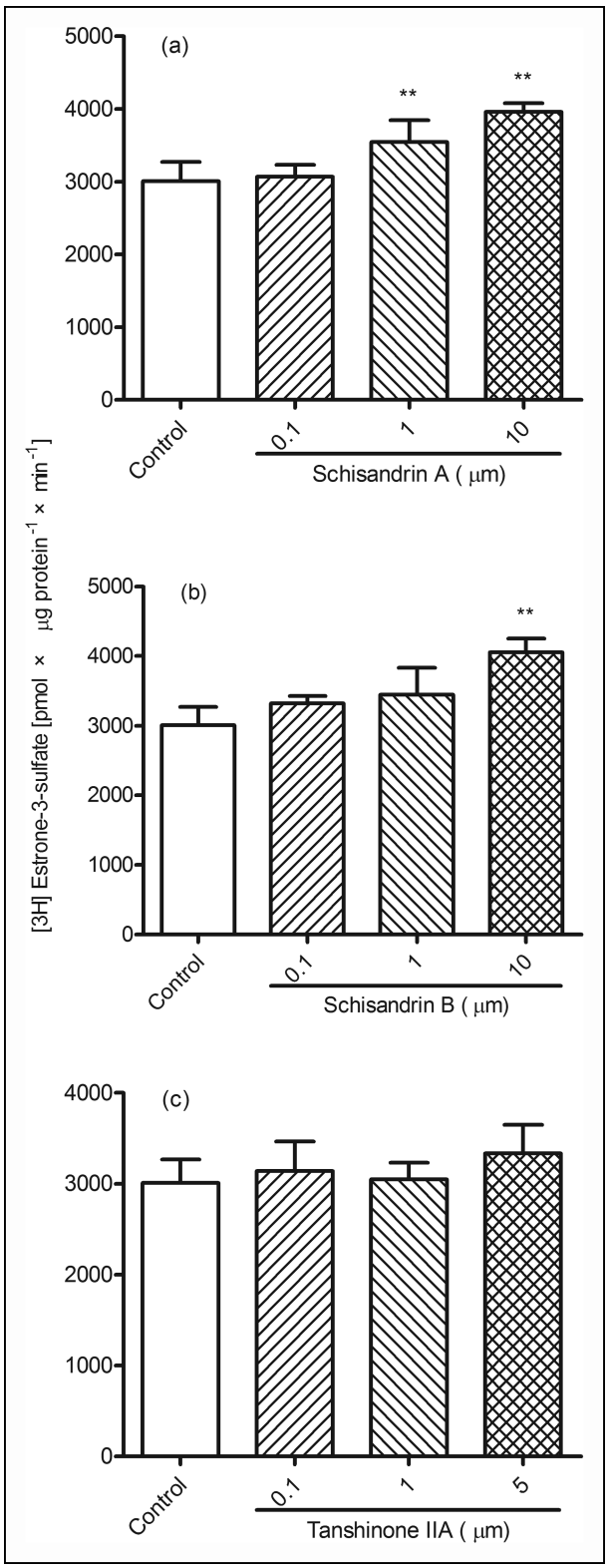

Fig. 3: Effect of schisandrin A and B on OATP1B1 transporter activity. (a): schisandrin A. (b): schisandrin B. (c): tanshinone IIA. Data are presented as the mean ± SD. Compared with the control group $^{*}P < 0.05$ and $^{**}P < 0.01$.

increased risk of statin-induced myopathy (Link et al. 2008; Mitka 2009). Improving transport function of OATP1B1 can increase the quantity of intracellular statins in liver cells and decrease the drug concentration in peripheral blood, which benefits for statin therapy in efficacy and safety. Both schisandrins A and B enhance transport function of OATP1B1 and could be used for the prevention of statin-induced myopathy.

The mechanism of regulatory OATP1B1 is complex. Hepatocyte nuclear factor (HNF1α) and nuclear receptor (pregnane X receptor, farnesoid X receptor, liver X receptorα) may be two major regulatory factors, which affect the expression or function of OATP1B1 (Jigorel et al. 2006; Sahi et al. 2006; Furihata et al. 2007; Meyer zu Schwabedissen et al. 2010). Rifampin is a Pregnane X Receptor (PXR) agonist in humans, which induces OATP1B1 mRNA expression approximately 2.3-fold to 2.4-fold in human primary hepatocytes (Jigorel et al. 2006; Sahi et al. 2006). However, OATP1B1 mRNA expression showed no apparent changes after treatment with rifampin in human hepatoma Huh-7 cells (Meyer zu Schwabedissen et al. 2010). Chenodeoxycholic acid (CDCA) significantly decreased OATP1B1 expression through repressing HNF1α (Jung and Kullak-Ublick 2003). CDCA increased OATP1B1 mRNA through activiting the farnesoid X receptor (FXR) (Meyer zu Schwabedissen et al. 2010). Therefore, we had no appropriate positive control in the study.

In conclusion, schisandrin A and B can induce OATP1B1 mRNA expression and enhance transporter activity. In clinical practice, the influence of schisandrin A and B on the absorption of OATP1B1 substrates needs further investigation.

4. Experimental

4.1. Chemicals

Schisandrin A and B (purity > 98%, HPLC determined) were provided by the National Institutes for Food and Drug Control (Beijing, China). Tanshinone IIA (purity > 98%, PLC determined) was provided by Prof. Lu-Qi Huang that work in China Academy of Chinese Medical Sciences (Beijing, China). [^{3}H] Estrone-3-sulfate (44Ci/mmol) was obtained from Perkin Elmer Life Sciences (Boston, MA, USA). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltertazolium bromide (MTT) was purchased from Promega (Madison, WI, USA). Trizol Reagent was purchased from Invitrogen (Carlsbad, CA, USA). Revertaid First Strand cDNA Synthesis kit was purchased from Fermentas Life Sciences (Helsingborg, Sweden).qPCR kit was obtained from TaKaRa Bio Inc (Tokyo, Japanese). Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade or higher.

4.2. Cell culture

Human hepatoma cell line HepG2 was from the American Type Culture Collection (ATCC). Cells were maintained in Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum and incubated at 37 °C in a 5% CO_2 atmosphere with medium change every two or three days.

4.3. Cytotoxicity assay of monomes extracted from medicinal plants

HepG2 cells (3.0×10^3 cells/well) were seeded in 96-well plates and cultured overnight. The cells were treated with different concentrations of schisandrin A and B and tanshinone IIA, which were dissolved in DMSO. The final concentration of DMSO in cell culture medium was 0.1% or less. After continued incubation for additional 48 h, the culture medium, which contained drugs, was removed. Then, MTT solution was added to each well for another 4 h of incubation at 37 °C. After removal of MTT solution, 100 μl of DMSO was added into each well for a gentle agitation on a shaker at room temperature for 10 min. The absorbance was measured at wavelength of 490 nm with a microplate reader. Viability was defined as the ratio of absorbance of treated cells to that of untreated cells) (Li et al. 2010). RNA isolation and real-time PCR analysis: HepG2 cells were seeded in 6-well plates at a density of 2×10^5 cells/well and incubated for 48 h. The medium was removed and replaced with DMSO, schisandrin A, schisandrin B, or tanshinone IIA for 48 h. Total RNA was isolated using the TRIzol reagent. Two micrograms of RNA was reverse transcribed according to the manufacturer's instructions. Real-time PCR was performed with the SYBR Green MasterMix system according to the manufacturer's instructions on a Mx3000P real-time PCR machine.

4.4. Measurement of human OATP1B1 transporter activity

The previous protocol was followed with minor modifications (Liu et al. 2009; Meyer zu Schwabedissen et al. 2010). HepG2 cells were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated for 48 h. The medium was removed and replaced with DMSO, schisandrin A, schisandrin

B, or tanshinone IIA for 48 h. Then, cells were washed using preheated Hank's balanced salt solution (HBSS) for three times and incubated in HBSS with [^{3}H] estrone-3-sulfate (50 nM) at the indicated concentrations for 10 min. Cells were washed in ice-cold HBSS for three times before lysed in 500 μl of lysis buffer containing 1 % sodium dodecyl sulfate (SDS) and centrifuged at 12,000 rpm for 15 min; 400 μL were used for the Beckman liquid scintillation analyzer. Protein concentration was quantified by the bicinchonic acid (BCA) kit.

4.5. Statistical analysis

All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Illinois, USA). One-way analysis of variance (ANOVA) was used for statistical comparisons. A p value of less than 0.05 was considered statistically significant.

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