

Chronic effect of Neurostan on the hepatic disposition of Fexofenadine in the isolated perfused rat liver

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Abstract

The overall purpose of this study was to investigate Neurostan Saint John's Wort (SJW) on the disposition of fexofenadine in the isolated perfused rat liver. Sprague-Dawley (SD) rats (n = 16) randomized into 3 groups, including: control, low dose (Hypericum, one type of neurostan) and high dose group. Each animal among these groups was pre-treated with either vehicle (ethanol in Milli Q water at 16 µg/ml) or low dose hypericum (150 mg/kg/day) or high dose hypericum (500 mg/kg/day), respectively for 14 consecutive days via gastric gavage. The administration volume was 1 ml/100g for all animals. Each rat liver was isolated and perfused in a recirculating system with medium containing fexofenadine at an initial concentration of 2000 ng/ml. The total amount (ng) of fexofenadine excreted into bile for the control vs. the low dose vs. the high dose group, was 141678 ± 32351 , 165270 ± 37340 and $222842 \pm 22996^*$ respectively, and the fexofenadine biliary clearance (ml/min) was 4.226 ± 0.955 , 4.855 ± 1.961 and $8.567 \pm 2.323^*$ respectively. Although, the ratio of liver to perfusate (L/P) was not significantly different, the ratio of bile to liver concentration (B/L) for the high dose group ($1.59 \pm 0.87^*$) was notably higher than that for the control group (0.82 ± 0.36). All together, it can be concluded that neurostan increases the hepatic p-glycoprotein (p-gp) thus raising the biliary clearance and the B/L ratio of the substrates (fexofenadine) transported by p-gp.

Key words: Keywords: Neurostan; P-glycoprotein (p-gp); Fexofenadine, Liver

Introduction

Saint John's Wort (SJW) is a complementary drug used in treating anxiety and depression, and several placebo-controlled clinical studies already confirmed its antidepressant effect (Capasso, Borrelli, Montanaro, Altieri, Capasso, & Izzo, 2005; Durr, Stieger, Kullak-Ublick, Rentsch, Steinert, Meier, & Fattinger, 2000). Neurostan, one of the most popular brand mainly containing SJW, is usually used in combination with other drugs such as fexofenadine. It was reported that SJW increased the expression of p-glycoprotein (p-gp) and

organic anion transporter protein (Oatp) in various tissues and organs (Durr et al., 2000; Hebert Mary, Park Jeong, Chen, Akhtar, & Larson Anne, 2004). Both *in vitro* and *in vivo* studies in animals indicate that SJW extract is not only a potential inducer of p-gp/ Oatp but also an inducer of CYP3A4 (Durr et al., 2000). Furthermore clinical studies prove that long-term (2 weeks) SJW administration in humans significantly induces intestinal and hepatic p-gp/ Oatp and CYP3A4 as well (Frye Reginald, Fitzgerald Sara, Lagattuta Theodore, Hruska Matthew, & Egorin Merrill, 2004). Both p-gp/ Oatp and CYP3A4 are hepatic disposal transporters which involved in substances clearance and biliary excretion and their upregulation would lead to an increase of clearance for some substances such as fexofenadine and consequently a decrease of its clinical efficacy (Johns, Brockmoller, Bauer, Maurer, Langheinrich, & Roots, 1999).

Some studies have reported that SJW would interfere with the pharmacokinetic parameters of some drugs as well as therapeutic effects (Tannergren, Engman, Knutson, Hedeland, Bondesson, & Lennernas, 2004) when they are used concurrently, but the research of SJW at different doses on the *in vivo* hepatic disposition of fexofenadine are rarely performed.

Fexofenadine, as a probe of p-gp and CYP3A4, is a non-sedate antihistamine using for the relief of allergy symptoms. Study on *mdrl* knockout mice validates that its bioavailability and clearance are dependent on p-gp expression (Cvetkovic, Leake, Fromm, Wilkinson, & Kim, 1999). Fexofenadine is actively transported across the sinusoidal membrane into hepatocytes via organic anion transporting protein (Oatp) and secreted into biliary canaliculi via p-gp (Meier, 1995; Milne, Larsen, Jorgensen, Bastlund, Stretch, & Evans, 2000). Therefore fexofenadine is a proper probe compound to examine the drug interference involved in hepatic transporting/excretion *in vivo*. Additionally, it is well documented that the ratios of liver to perfusate (L/P) and bile to liver (B/L) could be applied to as an index for the disposal of fexofenadine by p-gp and/or Oatp in which Oatp increased the L/B ratio while p-gp promoted the B/L ratio (Tong, Zhang, Ngo, & Davey, 2006).

This study is designed to explore the effects of neurostan on the hepatic p-gp activities in rats *in vivo*. Our experimental results show that neurostan increases the hepatic p-gp expression via which raises the biliary clearance and the B/L ratio of the substances clearance by p-gp such as fexofenadine.

Materials and Methods

Materials

Neurostan (Dr. Willmar Schwabe, special extract, each contains 300mg *Hypericum perforatum*, Germany) was obtained over the counter. Fexofenadine hydrochloride was purchased from Sigma-Aldrich □ Hoechst Marion Roussel Inc., Kansas, USA). Pentobarbitone sodium (60mg/ml Nembutal) was from Sino-Swed Pharmaceutical Corp Ltd (Dalian, China). Acetonitrile (HiPerSolv for HPLC) was bought from Fushun Shunnun Chemical Co Ltd (Liaoning, China). Male Sprague Dawley rats (weighing 250-380g) were obtained from the Animal center of Wuhan University (Luojia mountain, Wuhan, China).

Administration of Neurostan

The Animal Ethics Committee of Wuhan University approved this study. Rats were maintained in the animal facility of the Wuhan University under controlled temperature and humidity, with a 12-hour light/dark cycle. They had free access to food and water at all times.

Suspension was prepared by finely grounding the neurostan tablets with a glass pestle and mortar, then dampening the grounds with ethanol before diluting to the required volume with Milli-Q water (the ethanol content in each suspension was 16 µl/ml). The vehicle for the control group contained an equivalent volume of ethanol in Milli-Q water. All those preparations were protected from light and were used within 48 hours. Prior to treatment, SD rats were divided into 3 different groups randomly, control, low and high dose groups, and they were weighed and anaesthetized by halothane (4%)/oxygen (1.5L/min) mixture. The treatments were administered by gastric gavage, using a stainless steel, 4 inch 16G animal feeding needle. The rats were treated once daily (1ml/100g) for 14 consecutive days. The low dose group received 150mg/kg/day of *H. perforatum* (equivalent to 450 µg/kg/day of Hypericin, n = 6); the high dose group received 500mg/kg/day of *H. perforatum* (equivalent to 1500 µg/kg/day of Hypericin, n=5); the control group received vehicle (16 µg/ml ethanol in Milli-Q water, n = 5). All rats were treated proximately around 9am and the surgery for isolation of the liver was operated on 15th day after the final oral dose.

Perfusion procedure

Anaesthesia was induced by intraperitoneal injection of 60 mg/kg of sodium pentobarbitone prior to the surgery. The livers were perfused *in situ*, via the portal vein with a recirculating system at 37°C, described previously (Evans & Shanahan, 1995). The perfusion medium consisted of erythrocyte and albumin free Kreb's–Henseleit bicarbonate buffer, supplemented with glucose and sodium taurocholate (ref). The medium was prefiltered by vacuum filtration, using a 0.45µm filter membrane and the medium warmed to 37°C. The pH of the medium within the perfusion apparatus was maintained between 7.35-7.45 by bubbling with humidified carbogen (5% CO₂, 95% O₂). The perfusate was delivered to the liver at a flow rate of 30ml/min. After single pass perfusion of the liver with drug free medium for an equilibration period of 20min, 5ml of 100µg/ml fexofenadine stock solution was added to the perfusate (at t=0) to produce a initiate concentration of 2 µg/ml.

The liver was then perfused under recirculation conditions for a further 60min. The viability of each isolated perfused rat liver (IPRL) was confirmed by measuring the hepatic oxygen consumption (6-14mg/l), the bile flow rate ($\geq 5\mu\text{l}/\text{min}$), and by observing the gross appearance of the liver (uniform appearance and a pinkish-brown colour). The outflow perfusate samples were collected from the recirculation reservoir at 0, 1, 3, 5, 10, 15, 20, 25, 30, 40, 50 and 60min, and the bile samples were collected over each 10 minute interval from 0 to 60min. At the end of the perfusion, the liver was removed and weighed. The perfusate, bile and liver samples were stored at -20°C until analysed.

Sample analysis

A validated HPLC method involving UV detection was used to measure the concentrations of fexofenadine both in perfusate and bile. The HPLC system consisted of a Series 1100 series Autosampler, Series 1100 series Isocratic Pump, Series 1100 series Variable Wavelength Detector (Hewlett Packard, Germany), CR6A Chromatopac Integrator (Shimadzu, Japan); Column-Platinum EPS C₁₈ (100A5U, 250mm/4.6mm) and C₁₈ precolumn (Alltech Associates, USA). The detector wavelength was set to 225nm and the mobile phase was 0.024 M potassium dihydrogen orthophosphate: acetonitrile at a ratio of 58:42; pH was adjusted to 3.6 by using 0.1 M ortho-phosphoric acid. The retention time of fexofenadine under these conditions was found to be approximately 11min. The standard curves were prepared using the fexofenadine stock solution of 100 µg/ml with serial dilution to concentrations from 0.02 to 2.5µg/ml. Aliquots of the standard curve concentrations were stored at -20°C for use through the analytical process. Samples (the standard curve, perfusate, bile and livers) were thawed, vortexed, then mixed and centrifuged at 3000rpm before analysis. The standard curve in the liver homogenate was prepared by using the livers only through blank perfusion medium. The injection volume to HPLC was 100µl. The perfusate samples were injected undiluted while the bile samples were diluted 500 times using freshly blank perfusion medium gained from each test.

Data analysis

Pharmacokinetic parameters $t_{1/2}$, AUC, V_z , CL, and MRTINF were calculated by using Winnolin (WinNonLin Professional Version 4.1, Pharsight Corporation). Biliary pharmacokinetics were determined by the equation as follows:

$$CL_b = A_{e(0-60)} / AUC_{(0-60)}$$

Where $A_{e(0-60)}$ = total amount of fexofenadine excreted into the bile from time 0- 60 minutes; and $AUC_{(0-60)}$ = area under the perfusate concentration versus time curve from 0-60 minutes. Statistical comparisons of concentrations in perfusate; bile and liver homogenate among the three groups and the ratios of concentrations, and the L/P and B/L ratios were performed by single-factor analysis of variance separately; a *posteriori* comparisons were used to determine the effect of the dose of neurostan on pharmacokinetic parameters, $p < 0.05$ was considered statistically significant.

Results

The parameters in all the perfusions reflecting liver viability remained within normal range during the course of perfusion. The biliary flow rate during the perfusion experiment was all maintained above 5.0µl/min.

Perfusate analysis

The perfusate profiles were illustrated in Figure 1 as the mean perfusate concentrations of fexofenadine in the individual groups versus time. Although the concentrations of the high dose group were obviously lower than those in the other groups after 5 min-

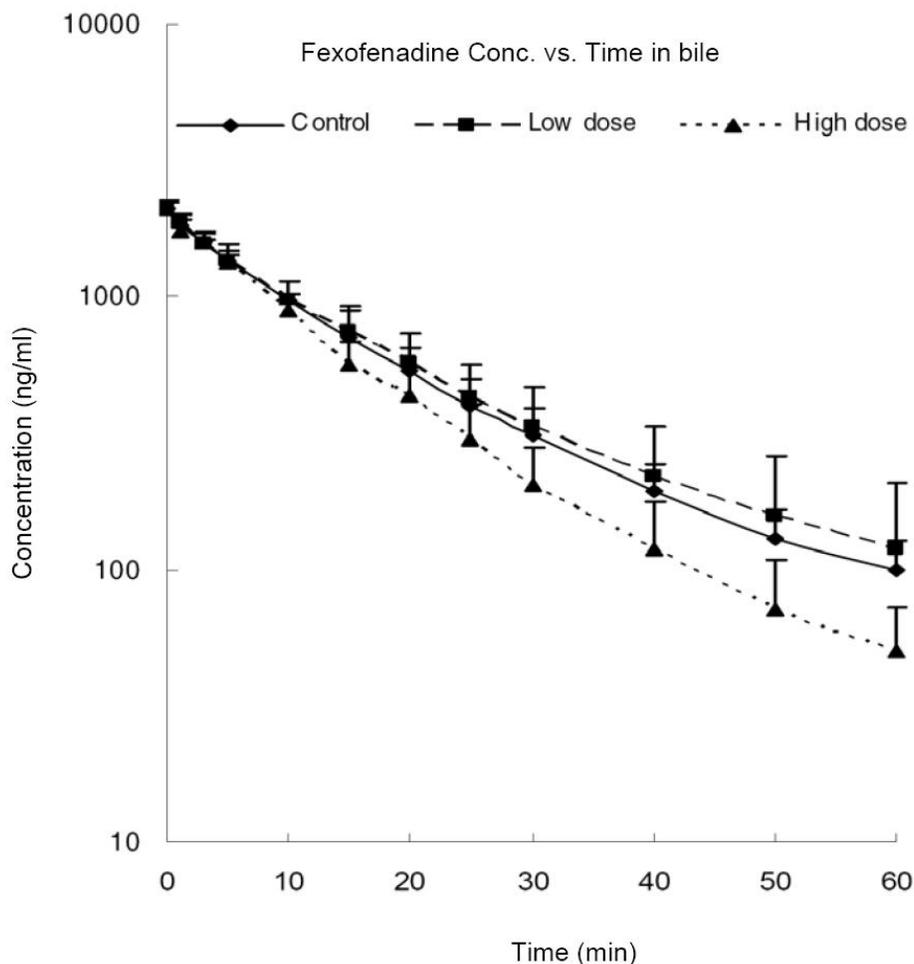


Figure 1. Comparison of Perfusate fexofenadine concentrations in 3 groups. Perfusate fexofenadine concentrations in 3 groups within 60 minutes. Results represent the mean \pm S.D. Control: n = 6, low dose group: n = 5, high dose group: n = 5.

utes as shown in the figure, no significant differences existed in $t_{1/2}$ (min), CL (ml/min) among these 3 groups as shown in Table 1. The ratio of liver to perfusate (L/P) was not significantly different (Table 2).

Bile analysis

The major novel finding of this study was shown in Figure 2 as the mean biliary excretion rate versus time. It showed that the rate of fexofenadine excreted into the bile in every minute during each collection period. For the time axis, middle was chosen to present the right collection interval. Significant differences were found in the three groups ($p = 0.04$). Moreover, the fexofenadine excretion rate is significantly higher in high dose group than in control ($p=0.004$) and low dose is ($p = 0.027$) as shown in Table 1. Furthermore, except clearance in perfusate and bile data, the clearance through other pathway could be calculated as: $CL_o = CL_t - CL_b$ in which CL_o , CL_t and CL_b represent other, total and significant differences were also observed in CL_b as shown in Table 1 among three groups. More specif-

Table1. Comparison of fexofenadine parameters in 3 different groups

Sample	$t_{1/2}$ lambda z (min)	Cl (observed) (ml/min)	CL_b (ml/min)	$A_{(0-60)}$ (ng)
Control	18.53 ± 9.24	15.19 ± 2.77	4.226 ± 0.955	141678 ± 32351
Low dose	22.37 ± 14.96	14.44 ± 4.44	4.855 ± 1.961	165270 ± 37340
High dose	11.23 ± 2.62	19.05 ± 3.71	8.567 ± 2.323*	222842 ± 22996*

Each value represents the mean ± s.d. Control: n=6, low dose group: n=5, high dose group: n=5. * p< 0.05.

cally, the p-value between the high and the control group for CL_b is 0.007, and between the high and the low is 0.014, the p-value among the three groups is 0.005(p <0.05). The bile flow rate in all these 3 groups generally remained stable throughout the experiment and was not significantly different biliary clearance, respectively. The other clearances for the control, low and high dose group are 10.97 ± 2.73 ; 9.62 ± 2.62 and 10.49 ± 1.67 respectively. No significant differences were found amongst CL_t , CL_o and $t_{1/2}$ (p =0.65).

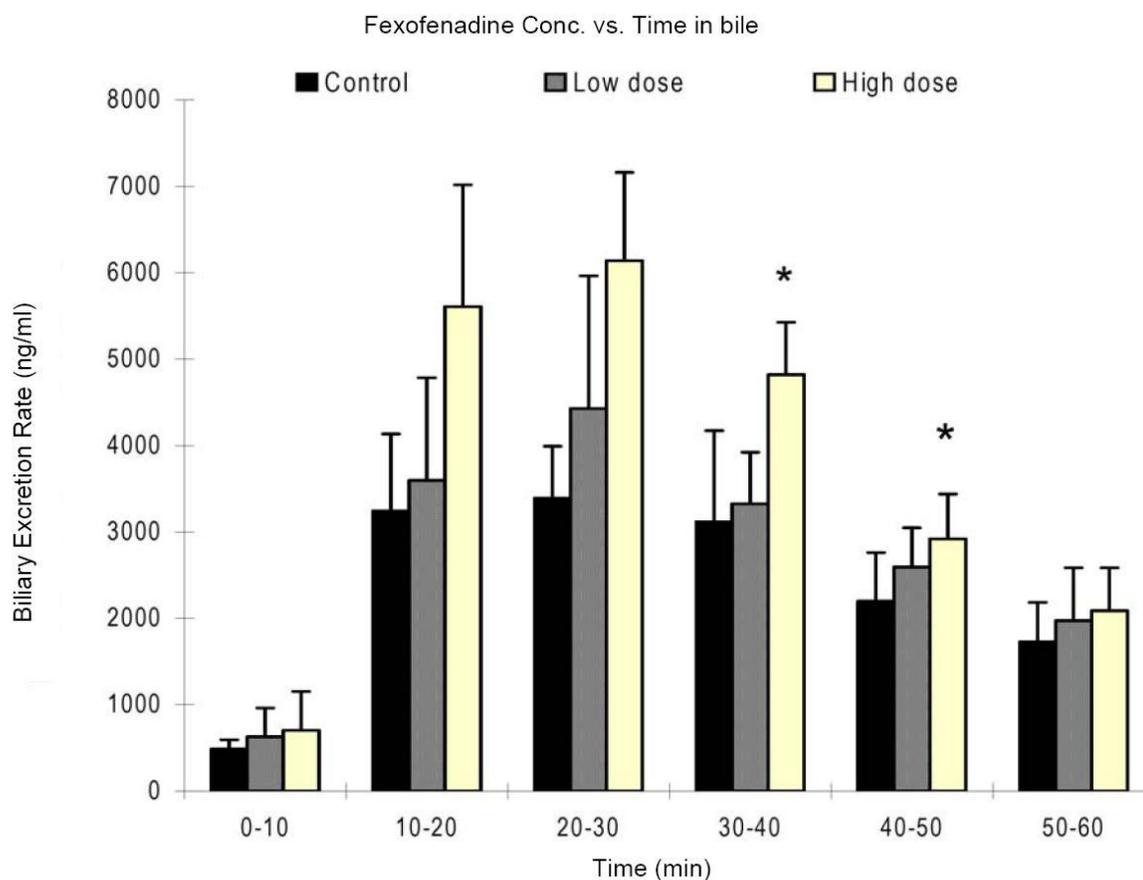


Figure 2. Comparison of Fexofenadine biliary excretion rate. Fexofenadine biliary excretion rate in the control group (n = 5), Neurostan low dose group (n = 6), and Neurostan high dose group (n = 5). Mean ± S.D.

Table 2. Ratios of fexofenadine in perfusate and liver and bile

Group	L/P	B/L
Control (n = 5)	237±36	0.82±0.36
Low dose (n = 6)	193±58	1.41±0.58
High dose (n = 5)	347±86	1.59±0.87*

B/L Ratio of concentrations of fexofenadine HCl in bile to liver.

L/P Ratio of concentrations of fexofenadine HCl in liver to perfusate.

* P<0.05 compared to control. Results are Mean ± S.D.

Liver homogenate analysis

The accumulated fexofenadine concentrations in rat liver homogenate were significantly different as shown in Table 1, with the p value among these three groups was 0.003. Moreover, the p value of the high dose to the control group was 0.0047, while the low dose to the control group was 0.0078. Of note, there was no significant difference between the low dose and the high dose group, although the ratio of bile to liver concentration (B/L) for the high dose group was notably higher than that for the control group (1.59±0.87* vs. 0.82 ± 0.36).

Discussion

This study shows that chronic administration of neurostan increased the hepatic accumulation, but increased the biliary clearance and the bile to liver concentration ratio (B/L) of fexofenadine. However, the clearance in perfusate or the liver to perfusate ratio (L/P) is not changed. Obviously as a marker of p-gp and other efflux transporters, fexofenadine must first traverse the sinusoidal (basolateral) membrane of the hepatocytes by passive diffusion and/or hepatic uptake of some transporters before it can be pumped into the bile by efflux transporters like p-gp (Zhang, Jie, Zhou, Cao, & Li, 2009; Zhou, Chan, Pan, Huang, & Lee Edmund, 2004; Zhou, Chan, Goh, Chan, Duan, Huang, & McLeod, 2005). As reported previously, main components of neurostan can induce p-gp and CYP3A4 enzymes upregulation both in rats and humans, and administration of Hyperforin extract to rats at an oral dose 1000mg/kg/day for 14 days resulted in a 3.8-fold increase of intestinal p-gp and a 2.5-fold increase of hepatic CYP 3A4 expression (Durr et al., 2000). Recent studies in vitro also show that pregnane X receptor (PXR or SXR) played a key role in activating p-gp (Ostberg, Bertilsson, Jendeberg, Berkenstam, & Uppenberg, 2002; Watkins Ryan, Maglich Jodi, Moore Linda, Wisely, Noble Schroeder, Davis-Searles Paula, Lambert Mill, Kliewer Steven, & Redinbo Matthew, 2003). PXR can activate the expression of the MDR1 gene and can be activated by a structurally diverse collection of xenobiotics and natural steroid (Zhou, Chan, Pan, Huang, & Lee, 2004). Hyperforin, the main component in neurostan, is found to be a potent agonist for PXR resulting in upregulation of p-gp (Mueller, Uehleke, Woehling, Petzsch, Majcher-Peszynska, Hehl, Sievers, Frank, Riethling, & Drewelow, 2004; Ostberg et al., 2002). The outcome of our study exhibits long-term administration of neurostan raises the hepatic p-gp, which is quite coherent with those researches (Durr et al., 2000).

The reason that the biliary excretion rate and the clearance in the high dose group are significantly higher than the other two groups as shown in Figure 2 is likely due to the induced hepatic p-gp is dose dependent, which further leads to both the biliary excretion rate and the clearance of fexofenadine in the high dose group to be increased approximately 2-folds. Obviously this effect is because that the neurostan increases or activates the p-gp in the canalicular membrane of rat liver (Durr et al., 2000; Tong et al., 2006; Zhang et al., 2009).

The bile flow rate is evaluated by comparing the total volume of bile produced during 60 minutes perfusion and the change of the bile flow rate for each collection interval. Statistical analysis shows there are no significant differences in the rat body weight or the bile flow rate among these three groups. The concentrations of fexofenadine in the liver homogenate for both the high and low dose groups are significantly lower than that in the control group. Probably it is due to the p-gp that is either increased or/and activated in the canalicular membrane of hepatocyte by neurostan via high dose of neurostan (concentration). As a result of increased expression of p-gp by neurostan, more substrates are pumped into the bile, resulting in the rise of the biliary clearance and the ratio of bile to liver concentration (B/L). Therefore, it is important to pay attention to pharmacokinetics alterations caused by combination therapy.

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