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Effect of Garlic, Gingko, and St. John's Wort Extracts on the Pharmacokinetics of Fexofenadine: A Mechanistic Study

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ABSTRACT

The aim of this study was to determine the effects of garlic and ginkgo herbal extracts on the pharmacokinetics of the P-glycoprotein (P-gp)/organic anion-transporting polypeptides (Oatps) substrate fexofenadine. Male rats were dosed orally with garlic (120 mg/kg), ginkgo (17 mg/kg), St. John's wort (SJW; 1000 mg/kg; positive control), or Milli-Q water for 14 days. On day 15, rats either were administered fexofenadine (orally or i.v.), had their livers isolated and perfused with fexofenadine, or had their small intestines divided into four segments (SI–SIV) and analyzed for P-gp and Oatp1a5. In vivo, SJW increased the clearance of i.v. administered fexofenadine by 28%. Garlic increased the area under the curve$_{0-\infty}$ and maximum plasma concentration of orally administered fexofenadine by 47% and 85%, respectively. Ginkgo and SJW had no effect on the oral absorption of fexofenadine. In the perfused liver, garlic, ginkgo, and SJW increased the biliary clearance of fexofenadine with respect to perfusate by 71%, 121%, and 234%, respectively. SJW increased the biliary clearance relative to the liver concentration by 64%. The ratio of liver to perfusate concentrations significantly increased in all treated groups. The expression of Oatp1a5 in SI was increased by garlic (88%) and SJW (63%). There were no significant changes in the expression of P-gp. Induction of intestinal Oatp1a5 by garlic may explain the increased absorption of orally administered fexofenadine. Ginkgo had no effect on the expression of intestinal P-gp or Oatp1a5. A dual inductive effect by SJW on opposing intestinal epithelial transport by Oatp1a5 and P-gp remains a possibility.

Introduction

The global market for herbal medicines has grown rapidly over the past decades and is estimated to be worth $80–100 billion (Abad et al., 2010). The concomitant administration of herbal medicines with conventional drugs may increase the risk of adverse effects from the drugs or decrease their efficacy (Tirona and Bailey, 2006; Izzo and Ernst, 2009). However, there appears to be a considerable lack of public awareness of the potential for herbal medicines to cause unwanted clinical outcomes and potentially life-threatening interactions with conventional drugs (Egan et al., 2011; Eichhorn et al., 2011; Licata et al., 2013). Garlic and ginkgo extracts are among the most popular herbal medicines used (Ude et al., 2013; Bayan et al., 2014). However, both have been shown to alter the plasma concentrations and/or pharmacological effects of various drugs. Administration of garlic extract to humans decreased exposure to the protease inhibitors, saquinavir and ritonavir, in plasma (Piscitelli et al., 2002; Gallicano et al., 2003). Intake of ginkgo altered the disposition of theophylline, nicardipine, and cyclosporine (Shinozuka et al., 2002; Yang et al., 2006; Tang et al., 2007) and has been associated with seizures in patients treated with the antiepileptic drugs, phenytoin and sodium valproate (Granger, 2001; Kupiec and Raj, 2005). The precise mechanisms behind the interactions are not fully understood, but the data suggest that interactions at the pharmacokinetic level involve changes in the activity and/or expression of drug-metabolizing enzymes and/or transporters (Abad et al., 2010; Hajda et al., 2010).

Both uptake and efflux transporters play an important role in determining the absorption and disposition of an orally administered drug. P-glycoprotein (P-gp; also known as *ABCB1*) and organic anion-transporting polypeptides (OATPs/*SLC21A*; Oatps/*Slc21a* in rodents) exhibit significant overlap in substrate specificity and colocalize in tissues. They are a likely choice for research investigating the mechanisms of interaction because of their key roles in the disposition and clearance (CL) of drugs. To date a number of studies have investigated the effects of garlic and ginkgo extracts on the function of P-gp and OATP/Oatps, and although there is growing evidence that suggests both extracts may well alter the function of P-gp and OATP/Oatps, conflicting results that demonstrate inhibition, no effect or induction (Foster et al., 2001; Gallicano et al., 2003; Robertson et al., 2008; Li et al., 2009; Hajda et al., 2010), and the lack of detailed analysis preclude meaningful conclusions.

The aim of this paper was to investigate the impact of chronic administration of garlic and ginkgo extracts on P-gp– and Oatp-mediated transport in rats. Fexofenadine was used as a probe substrate. Fexofenadine is metabolized only to a minor degree in humans and rats. The major organ responsible for its elimination is the liver via biliary CL (Lippert et al., 1995; Russell et al., 1998; Milne et al., 2000; Kamath et al., 2005). Fexofenadine is a substrate for both intestinal and hepatic P-gp and Oatps (Milne et al., 2000; Kikuchi et al., 2006; MacLean et al., 2010). This study was conducted in three stages: first, the impact of the two herbal extracts on the pharmacokinetics of fexofenadine was studied

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ABBREVIATIONS: Ae$_{0-60}$, cumulative amount excreted into bile from time 0 to 60 minutes; AUC, area under the curve; B/L, bile to liver; CAR, constitutive androstane receptor; CL, clearance; CL$_{b,l}$, biliary clearance with respect to the concentration in liver; CL$_{b,p}$, biliary clearance with respect to the concentration in perfusate; L/P, liver to perfusate; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein; SJW, St. John's wort.

in rats; and, from the observations of this, two further studies were performed to evaluate their effect on the disposition of fexofenadine in the isolated perfused liver, and on the expression of two transporting proteins, P-gp and oatp1a5, in intestinal segments.

St. John's wort (SJW; *Hypericum perforatum*) is a popular herbal product used as an alternative to conventional antidepressants for the treatment of mild to moderate depression. Work from our laboratory has demonstrated that its oral administration (1000 mg/kg/d) for 14 days increases the hepatic transport of fexofenadine into bile (Turkanovic et al., 2009). Other research has established that SJW is a potent inducer of intestinal P-gp (Durr et al., 2000; Kageyama et al., 2006). Hence, a group of rats treated with SJW served as a positive control for induction of P-gp. The commercial brands of garlic and ginkgo extracts were selected on the basis of their popularity in the market, as determined by an informal survey of several local pharmacies in Australia. Taking into account the differences in metabolic CL and body weight between rats and humans, the oral doses of both extracts were designed to be about 10-fold of those per day to humans (Yoshioka et al., 2004). Potent inducers can achieve substantial induction of P-gp and Oatps within 4 days (Rausch-Derra et al., 2001; Kageyama et al., 2006). In this study, rats were pretreated with the extracts for 14 days to ensure maximum induction potential.

Materials and Methods

Chemicals

Fexofenadine HCl was purchased from Toronto Research Chemicals (North York, ON, Canada). Tablets containing St. John's wort SJW extract (Kira LI-160 extract, 300 mg, standardized to contain 900 μg hypericin; Lichtwer Pharma AG, Berlin, Germany) were purchased from Thompson's Nutrition (Auckland, New Zealand). Products containing extracts of garlic (Garlix, 400 mg, standardized to contain 10 mg alliin, equivalent 4.6 mg allicin; Blackmores, NSW, Australia) and ginkgo (Ginkgoforte, 40 mg, standardized to contain 10.7 mg ginkgo flavonol glycosides and 2.7 mg ginkgolides and bilobalide; Blackmores) were purchased from a local pharmacy. Other chemicals were of analytical grade and used as supplied commercially.

Animals

All animal procedures were approved by the Institute of Medical and Veterinary Science Animal Ethics Committee (Adelaide, South Australia). Male Sprague–Dawley rats (300–350 g) were obtained from the Institute of Medical and Veterinary Science (Adelaide, South Australia). They were housed separately in plastic cages in the animal facility of the University of South Australia under controlled conditions (23°C, 12-hour light/dark cycle).

In Vivo Study

In order for the fexofenadine to be administered orally or i.v. after the herbal treatment, rats were divided randomly into eight groups (five to six rats/group), as follows: two control, two garlic, two ginkgo, and two SJW-treated groups. Suspensions of the garlic, ginkgom, and SJW extracts were prepared immediately before dosing by grinding the tablets and diluting to the required volume with Milli-Q water. Rats were dosed orally (10 mL/kg) with garlic (120 mg/kg), ginkgo (17 mg/kg), or SJW (1000 mg/kg) by intragastric gavage once daily for 14 days; control rats received Milli-Q water only. On day 14, the jugular vein was cannulated with silicone rubber tubing. Fexofenadine (25% dimethylsulfoxide: 75% water) was administered orally or i.v. on day 15. All rats were fasted overnight (access to food was restored after the 6-hour blood sample was obtained). The oral dose of fexofenadine (100 mg/kg) was administered via intragastric gavage; the i.v. dose (10 mg/kg) via the penile vein. Blood (200 μL) was collected via the jugular vein at 0 (predose), 5, 10, 15, and 30 minutes and 1, 2, 4, 6, 8, and 24 hours after the dose. Blood taken was replaced by an equivalent volume of sterile saline. Samples were centrifuged (15,000*g*, 5 minutes) immediately, and the plasma was stored at −80°C until analysis.

Liver Perfusion

Rats were divided randomly into four groups (seven rats/group) and dosed orally with garlic, ginkgo, SJW, or Milli-Q water once daily for 14 days as per above. On day 15, rats were anesthetized with 60 mg/kg sodium pentobarbitone (Nembutal 60 mg/ml; Boehringer Ingelheim, North Ryde, NSW, Australia). Livers were prepared for perfusion in situ, as described previously (Milne et al., 2000). In brief, the liver was perfused (30 mL/min, single pass manner) via the hepatic portal vein with oxygenated drug-free Krebs-bicarbonate buffer for an equilibration period of 15 minutes. Subsequently, the perfusion was switched to recirculating mode, and the liver was perfused for 1 hour with fexofenadine HCl (2000 ng/mL). Perfusate samples (1 mL) were collected from the cannulated vena cava at 0, 1, 3, 5, 10, 15, 20, 25, 30, 40, 50, and 60 minutes after the addition of fexofenadine HCl. All bile was collected over six 10-minute intervals. Livers were collected and weighed at the end of each perfusion experiment. Liver viability was confirmed by assessing its gross appearance (evenly perfused), bile flow (>5 μL/min), and the recovery of venous perfusate (>98% of the volume of inflowing perfusing medium) (Qi et al., 2010).

Quantitation of the Expression of Intestinal P-gp and Oatp1a5

Rats were divided randomly into four groups (four to six rats/group) and dosed orally with garlic, ginkgo, SJW, or Milli-Q water once daily for 14 days, as per above. On day 15, rats were anesthetized (isoflurane/oxygen mix), and the small intestine was removed. The expression of drug-transporting proteins can differ along the intestine. For example, P-gp increases from the proximal to the distal region (MacLean et al., 2008). Therefore, rather than treat the entire small intestine as one single sample, it was divided into four equal segments (~25 cm each) and analyzed for differential expression of Oatp1a5 and P-gp along its length. The proximal segment, starting from the pylorus, was designated number I, whereas the most distal segment, close to the ileocecal valve, was designated number IV. The intestinal segments were flushed with ice-cold saline, slit along their entire length, and laid on a chilled ceramic plate. Mucosal tissue was obtained from each segment by scraping with a microscope slide. Mucosal samples were weighed and homogenized with T-PER (20 mL/g; Pierce, Rockford, IL). The homogenates were centrifuged at 3000*g* for 15 minutes, and the resulting supernatant was centrifuged at 27,000*g* for 30 minutes. The pellets were resuspended in buffer containing 300 mM mannitol and 40 μg/mL phenylmethylsulfonyl fluoride (pH 7.5) (Ghanem et al., 2006; Kageyama et al., 2006). Protein concentrations in membrane preparations were measured with the bicinchoninic acid protein assay (Pierce). The following experiments were performed in triplicate for each sample. For Oatp1a5 analysis, 100 μg total protein was used for each segment. For P-gp analysis (to avoid overload), different amounts of total protein were used for each of the four segments (I = 50 μg; II = 30 μg; III = 20 μg; IV = 15 μg). Samples were precipitated with ice-cold acetone, resuspended in sample loading buffer (equal mixture of 8 M urea, sample buffer, and reducing agent), heat-denatured (30 minutes at 56°C), and loaded onto precast 4–12% gradient polyacrylamide gels (Invitrogen, Mulgrave, VIC, Australia). Proteins were resolved by electrophoresis at 130 V for 1.5 hours and transferred to polyvinylidene difluoride membranes at 35 V for 1.5 hours. Membranes were then blocked with 5% dry skim milk in Tris-buffered saline containing 0.01% Tween 20 for 1 hour at room temperature (20–23°C). Blocked membranes were incubated overnight at 4°C with anti–P-gp (Alexis Biochemicals, Grunberg, Germany) or anti-Oatp1a5 (Santa Cruz Biotechnology, Dallas, TX) primary antibodies (diluted 1:100 in blocking solution). Bound antibodies were detected with goat anti-mouse and donkey anti-goat antibodies (Sigma-Aldrich, St. Louis, MO) (diluted 1:2000 in Tris-buffered saline containing 0.01% Tween 20). β-Actin (1:1000) was used as the loading control. Protein bands were visualized by enhanced chemiluminescence, according to the manufacturer's instructions, and photographed using a FluorChem 8900 imager. Relative expressions were quantified densitometrically using AlphaView software and calculated by normalization to the reference bands of β-actin.

Measurement of Fexofenadine Concentrations in Plasma, Perfusate, Bile, and Liver

Fexofenadine concentrations in plasma were measured by liquid chromatography coupled with tandem mass spectrometry. The system consisted of a LC-10AD binary pump, DGU-14A degasser, SIL-HTC autosampler (all from

Shimadzu, Kyoto, Japan), and API 3000 mass spectrometer (Applied Biosystems, Foster, Canada). Plasma samples (100 μL) were vortex-mixed with 5 μL internal standard (5000 ng/mL levocabastine in water) and 900 μL ethyl acetate and centrifuged (4°C, 1500*g*, 10 minutes). After centrifugation, supernatants were transferred to a clean Eppendorf tube and dried under nitrogen at 37°C. Residues were reconstituted in 50 μL mobile phase (methanol and water; 48:52, v/v) and injected (30 μL) onto a GraceSmart C18 (3 μm, 50 mm × 2.1 mm; Grace Davison Discovery Science, Chicago, IL) column preceded by a C18 guard column (4.0 × 2.0 mm; Phenomenex, Torrance, CA). The mobile phase (methanol and water; 48:52, v/v) was delivered isocratically at a flow rate of 0.3 mL/min. Ions were generated using electrospray ionization and detected in the positive-ion mode. Multiple reaction monitoring was used to detect the m/z 502.5/466.5 and 502.5/484.5 transitions for fexofenadine and the 421.2/174.2 transition for levocabastine. Standard curves were linear over the range 1–1000 ng/mL. Plasma samples with concentrations higher than the upper limit of quantification were diluted into the linear range with blank rat plasma. The limit of quantification of the assay was defined as the lowest concentration of the standard curve sample that could be measured with an intraday accuracy and precision within 15% using six replicates. Concentrations of fexofenadine in the perfusate, bile, and liver were measured using an adaptation of a previously published high-pressure liquid chromatography method (Milne et al., 2000; Turkanovic et al., 2009). Perfusate and bile (diluted 1:500 with drug-free perfusate) were injected (SIL-10AD; Shimadzu) in a volume of 100 μL onto a platinum EPS C_{18} analytical column (100Å, 5 μm, 250 mm × 4.6 mm) preceded by a C_{18} precolumn (Alltech, Woodridge, IL). Fexofenadine was eluted at about 10 minutes using a mobile phase of acetonitrile and 0.024 M potassium dihydrogen orthophosphate (42:58 v/v, pH adjusted to 3.6 using 1 M orthophosphoric acid) pumped at 1 mL/min (LC-10AT; Shimadzu), and quantified by UV absorbance (SPD-10AV UV; Shimadzu) at 225 nm. Calibration curves were constructed without weighting from the peak heights of fexofenadine versus the concentrations of fexofenadine. The curves were linear over the range 25–2000 ng/mL.

Weighed livers were homogenized in an equal volume of water. Homogenate (0.7 mL) was mixed with an equal volume of acetonitrile, vortex-mixed, and centrifuged at 1000*g*. The supernatant was passed through a 0.45-μm syringe filter, and 100 μL was injected onto the high-pressure liquid chromatography column. Calibration curves were constructed without weighting from the peak-height of fexofenadine versus the concentrations of fexofenadine. Standard curves were linear over the range 2,000–12,000 ng/mL. The accuracy of the quality control samples spanning the calibration concentrations was within 15%.

Pharmacokinetic and Statistical Analysis

In Vivo Study. Noncompartmental analysis, using the concentrations of fexofenadine in plasma, was performed using WinNonlin (Version 4.0; Pharsight, Mountain View, CA). The calculated pharmacokinetic parameters included half-life, CL, and volume of distribution at steady state. Area under the curve $(AUC)_{0-\infty}$ was calculated using the linear trapezoidal rule with extrapolation beyond the last measured concentration using the terminal rate constant. Mean bioavailability was calculated from the dose-normalized values of $AUC_{0-\infty}$ obtained from i.v. and oral administrations.

Liver Perfusion Study. AUC from 0 to 60 minutes (AUC_{0-60}), area from 0 to infinity ($AUC_{0-\infty}$), and CL from the perfusate were calculated using a non-compartmental i.v. bolus model in WinNonlin. The $AUC_{0-\infty}$ of fexofenadine was calculated using the linear trapezoidal method, and used to calculate CL. The cumulative amount excreted into bile from time 0 to 60 minutes (Ae_{0-60}) was the summed products of the biliary volume and concentration of fexofenadine during each collection interval. Biliary CL with respect to the concentration in perfusate ($CL_{b,p}$) was obtained by dividing Ae_{0-60} by AUC_{0-60}. Biliary CL with respect to the concentration in liver ($CL_{b,l}$) was the quotient of the rate of excretion of fexofenadine into bile at the 50- to 60-minute collection interval and its concentration in the liver at 60 minutes. Concentrations in perfusate, bile, and the liver at 60 minutes were used to calculate the ratios of the concentrations of fexofenadine in liver to perfusate (L/P; reflecting uptake across the sinusoidal membrane) and bile to liver (B/L; reflecting efflux across the canalicular membrane).

All data were tested for normality and homogeneity of variance (SPSS 19.0 for Windows, Chicago, IL). When the normality test failed, data were log-transformed before statistical analysis. Single-factor analysis of variance (SPSS 19.0 for Windows) was used to test for differences between the experimental groups. Where significant differences were identified, post hoc analysis was performed using the least significant differences test. Differences between groups were considered statistically significant at $p < 0.05$.

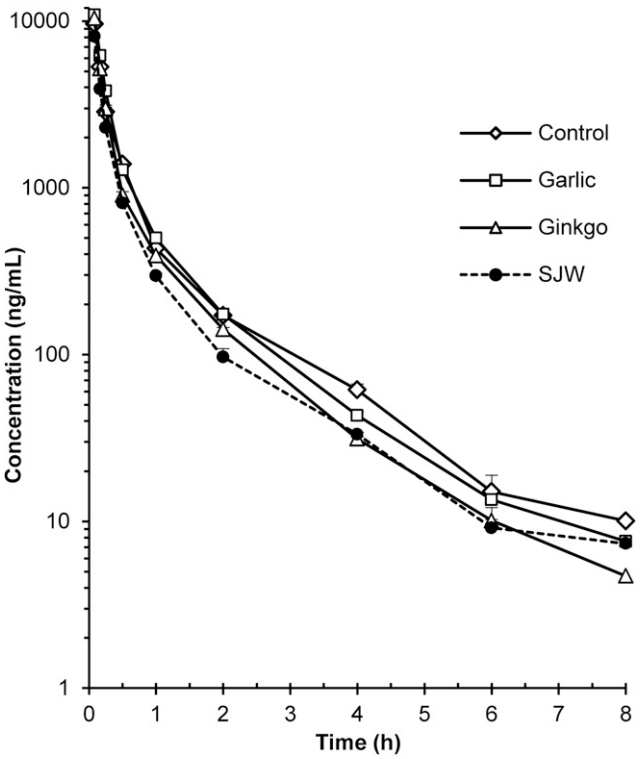

Fig. 1. Concentration–time profiles of fexofenadine in plasma. Rats, treated previously with garlic (120 mg/kg), ginkgo (17 mg/kg), and SJW (1000 mg/kg) for 14 days, received a bolus i.v. dose of 10 mg/kg fexofenadine. Each data point represents the mean ± S.E.M. ($n = 6$).

Results

In Vivo Study. Figure 1 shows the mean concentrations of fexofenadine in plasma as a function of time following i.v. administration to the four groups of rats. The corresponding pharmacokinetic parameters are presented in Table 1. Administration of SJW significantly ($p < 0.05$) increased the CL of i.v. administered fexofenadine by 28% compared with control. Pretreatment with garlic and ginkgo had no effect. Figure 2 shows the mean concentrations of fexofenadine in plasma following oral administration to the four groups of rats. The corresponding pharmacokinetic parameters are summarized in Table 2. Pretreatment with garlic significantly ($p < 0.05$) increased the $AUC_{0-\infty}$ and maximum plasma concentration of orally administered fexofenadine by 47% and 85%, respectively. There were no significant ($p > 0.05$)

TABLE 1

Pharmacokinetic parameters for fexofenadine in garlic-, ginkgo-, and SJW-treated rats following an i.v. administration of fexofenadine (10 mg/kg); data represent mean ± S.E.M. ($n = 6$)

Parameter	Control	Garlic	Ginkgo	SJW
$AUC_{0-\infty}$ (min × μg/mL)	223 ± 14	246 ± 24	219 ± 12	173 ± 12*
$t_{1/2}$ (h)	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
CL (mL/min)	15.4 ± 1.0	14.4 ± 1.5	15.9 ± 1.0	19.7 ± 1.7*
Vss (L/kg)	0.56 ± 0.1	0.44 ± 0.1	0.43 ± 0.1	0.58 ± 0.1

$t_{1/2}$, half-life; Vss, volume of distribution at steady state.
*$p < 0.05$ versus control group.

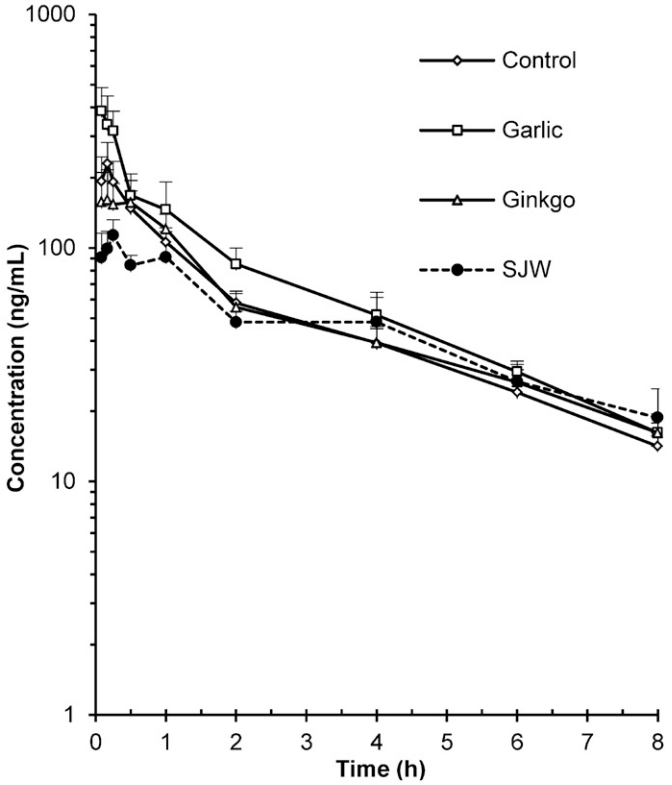

Fig. 2. Concentration–time profiles of fexofenadine in plasma. Rats, treated previously with garlic (120 mg/kg), ginkgo (17 mg/kg), and SJW (1000 mg/kg) for 14 days, received an oral dose of 100 mg/kg fexofenadine. Each data point represents the mean ± S.E.M. (n = 5).

changes in the extent of oral absorption of fexofenadine following administration of SJW and ginkgo. The percentage of the $AUC_{0-\infty}$ extrapolated beyond the last measured concentration was less than 1.5% for all values.

Liver Perfusion Study. Concentrations of fexofenadine in the perfusate are shown in Fig. 3. The CL from perfusate was increased significantly ($p < 0.05$) by ginkgo (41%) and SJW (48%), but not garlic ($p > 0.05$, Table 3). Pretreatment with SJW, garlic, and ginkgo significantly ($p < 0.05$) increased the Ae_{0-60} by 146%, 48%, and 65%, respectively, over the control group (Fig. 4; Table 3); SJW, garlic, and ginkgo significantly ($p < 0.05$) increased the $CL_{b,p}$ by 234%, 71%, and 121%, respectively, over the control group (Table 3). The L/P was increased significantly ($p < 0.05$) by all treatments. There were no statistically significant ($p > 0.05$) differences in the value of B/L between the groups. There were no significant ($p > 0.05$) changes in $CL_{b,l}$ following garlic and ginkgo administration, but it was increased significantly ($p < 0.05$) in the SJW-treated group (Table 3).

Quantitation of the Expression of P-gp and Oatp1a5 in Intestinal Segments (SI-IV). The expression of Oatp1a5 in SI was increased significantly ($p < 0.05$) from control by garlic (88%) and SJW (63%) (Fig. 5A). There were no significant changes ($p > 0.05$) in the expression of P-gp following administration of SJW, garlic, and ginkgo (Fig. 5B).

Discussion

SJW increased the CL of i.v. administered fexofenadine significantly (28%; Table 1). The major contributor to this increase is most likely induction of one or more hepatic transporters responsible for its elimination from plasma into bile, which would concur with results from a previous study assessing the disposition of fexofenadine in perfused livers from SJW-treated rats (Turkanovic et al., 2009). SJW did not have a significant effect on the pharmacokinetics of oral fexofenadine. Similarly, a previous clinical study found no effect from SJW on the pharmacokinetics of oral fexofenadine (Wang et al., 2002). One plausible explanation could be a dual inductive effect on opposing intestinal transport by Oatp and P-gp. In rats, fexofenadine is a substrate for P-gp, Oap1a1, Oatp1a4, and Oatp1a5 (Cvetkovic et al., 1999; Kikuchi et al., 2006). Intestinal P-gp and Oatp1a5 are both located on the apical membrane of enterocytes: the first transporting intracellular substrate into the intestinal lumen, and the second facilitating uptake from the intestine (Walters et al., 2000; Fu and Arias, 2012). Furthermore, augmented hepatic CL could also have contributed to the observation: P-gp in the liver and intestine reducing exposure after an oral dose and Oatp1a5 in the intestine increasing exposure. Meanwhile, augmented hepatic CL would account for the reduced exposure to fexofenadine after an i.v. dose.

Garlic did not alter the pharmacokinetics of fexofenadine administered i.v., but there were respective increases of 47% and 85% in $AUC_{0-\infty}$ and maximum plasma concentration of fexofenadine after oral administration (Table 2). Fexofenadine is mainly absorbed in the upper regions of the gastrointestinal tract (MacLean et al., 2010). An earlier study by MacLean et al. (2010) has suggested that there are no significant differences in the levels of Oatp1a5 mRNA across the small intestine. However, the pattern of expression of Oatp1a5 protein along the intestine has not been studied previously; in the present study, its expression in control rats (Fig. 5A) was significantly lower in segment I as compared with segments II and III. P-gp is most abundant in the lower regions of the small intestine in rats and humans, whereas the quantity in the upper regions is low (Mouly and Paine, 2003; Ghanem et al., 2006; MacLean et al., 2008) and more easily saturable by potentially high concentrations created from rapid absorption of the oral solution. Therefore, induction of intestinal Oatp1a5 by garlic may have caused the increased rate and extent of absorption of fexofenadine.

There was no effect of ginkgo on the pharmacokinetics of fexofenadine. There are two possible explanations: no impact on the expression

TABLE 2

Fexofenadine pharmacokinetics in garlic-, ginkgo-, and SJW-treated rats following an oral administration of fexofenadine (100 mg/kg); data represent mean ± S.E.M. (n = 5)

Parameter	Control	Garlic	Ginkgo	SJW
$AUC_{0-\infty}$ (min × μg/mL)	28.8 ± 3.8	42.3 ± 6.2*	29.2 ± 3.8	25.9 ± 3.4
$t_{1/2}$ (h)	2.4 ± 0.3	2.7 ± 0.4	2.5 ± 0.2	2.7 ± 0.7
C_{max} (min)	240 ± 49	444 ± 89*	191 ± 51	133 ± 22
T_{max} (min)	9 ± 1	8 ± 2	17 ± 5	24 ± 10
F (%)	1.29 ± 0.17	1.72 ± 0.25	1.33 ± 0.17	1.50 ± 0.20

C_{max}, maximum plasma concentration; F, bioavailability; $t_{1/2}$, half-life; T_{max}, time to maximum plasma concentration.
*$p < 0.05$ versus control group.

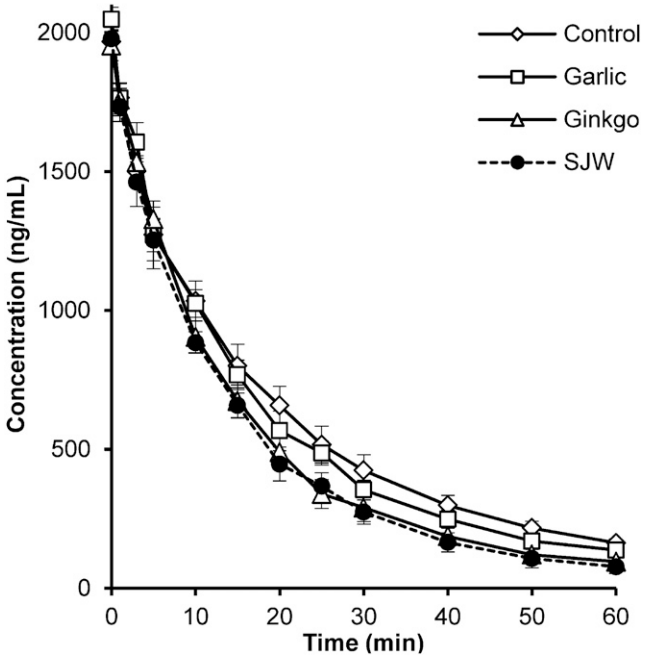

Fig. 3. Concentration–time profiles of fexofenadine in outflowing perfusate. Livers from rats, treated previously with garlic (120 mg/kg), ginkgo (17 mg/kg), or SJW (1000 mg/kg) for 14 days, were isolated and perfused with fexofenadine at an initial concentration of 2000 ng/mL for 1 hour. Each data point represents the mean ± S.E.M. (n = 7).

of Oatp and P-gp, or opposing effects on both of them. A previous clinical study by Robertson et al. (2008) found no effect on the pharmacokinetics of oral fexofenadine after 2 weeks of administering ginkgo extract. An in vitro study, using human hepatocytes, found that ginkgo induced the expression of multiple drug-metabolizing enzymes and transporters, including P-gp, through selective activation of the pregnane X receptor, constitutive androstane receptor (CAR), and the aryl hydrocarbon receptor (Li et al., 2009).

In the liver perfusion study, an increase in $CL_{b,p}$ of fexofenadine (Table 3) by SJW is an indication of enhanced carrier-mediated transport from perfusate into bile. The $CL_{b,p}$ of fexofenadine is a composite of its sequential transport across the sinusoidal and the canalicular membranes into bile. $CL_{b,l}$, a CL that estimates excretion across the canalicular membrane relative to concentrations within liver tissue (Turkanovic et al., 2009), was increased significantly by SJW. The significant increase in $CL_{b,l}$ together with the trend for an increase in the B/L ($p < 0.1$) ratio (Table 3) suggests that SJW increased the activity of a transporting protein responsible for the carriage of fexofenadine from hepatocytes into bile (Milne et al., 2000; Tong et al., 2006). The L/P ratio, a parameter that reflects uptake across the sinusoidal membrane (Milne et al., 2000; Tong et al., 2006), was significantly increased by SJW (Table 3). An increased L/P suggests induction of Oatp, whereas an increase in $CL_{b,l}$ and B/L is consistent with induced P-gp activity (Milne et al., 2000; Turkanovic et al., 2009). This is the first study to suggest an inductive effect of SJW on the activity of hepatic Oatp.

Garlic and ginkgo increased the $CL_{b,p}$ of fexofenadine significantly, but the B/L ratio and $CL_{b,l}$ were unchanged (Table 3). Together, these observations indicate that canalicular transport was not affected by treatment with garlic or ginkgo. The L/P ratio was significantly increased by both treatments, implying an increased uptake across the sinusoidal membrane. Although $CL_{b,p}$ was increased significantly by both, only ginkgo increased the total CL, not garlic (Table 3). An increase in $CL_{b,p}$, but no change in CL, has been observed in previous experiments (Milne et al., 2000; Turkanovic et al., 2009) and most likely reflects a delay in the transfer of fexofenadine from perfusate to bile, meaning that the increase in $CL_{b,p}$ was not sufficient to have an impact on overall CL. There were no changes in the pharmacokinetics of i.v. fexofenadine in rats treated with garlic and ginkgo. Fexofenadine has a relatively high hepatic extraction ratio (Matsushima et al., 2008; Swift et al., 2009). It is possible that, even though an increased efficiency in the hepatic uptake of fexofenadine was observed in garlic- and ginkgo-treated rats, the overall changes in efficiency of elimination by the liver arising from administration of these two extracts were not of sufficient magnitude to have an effect in vivo (Rowland and Tozer, 2010). The impact of SJW on the hepatic transport was greater, and, hence, changes in vivo were detected.

Figure 5A shows that garlic induced the expression of Oatp1a5 (segment I). Therefore, induction of intestinal Oatp1a5 by garlic most probably explains the increased absorption of orally administered fexofenadine in vivo. Garlic had no effect on the expression of intestinal P-gp (Fig. 5B). This is in contrast to a human study that reported an increase of 31% in the expression of intestinal P-gp from long-term administration (3 weeks) of garlic extract, but found no effect on the pharmacokinetics of pravastatin (substrate for hepatic OATP1B1) (Hajda et al., 2010). Hajda et al. (2010) concluded that garlic extract does not have an effect on the expression and function of OATP1B1. The authors did not measure the expression of OATP1B1, and pravastatin is also known to be a substrate of MRP2 (Kivistö and Niemi, 2007). Hence, an effect of garlic extract on transporters such as this cannot be excluded. Another reason for the different outcomes could be the use of different species (i.e., humans versus rats).

SJW induced intestinal Oatp1a5 (Fig. 5A), but had no significant effect on the expression of P-gp (Fig. 5B). However, the possibility that SJW has an effect on intestinal P-gp cannot be completely excluded in this study. SJW is a well-known inducer of both rat and human intestinal

TABLE 3

Influence of garlic, ginkgo, and SJW administration on the pharmacokinetic parameters of fexofenadine in the isolated perfused rat liver (mean ± S.E.M., n = 7)

Parameter	Control	SJW	Ginkgo	Garlic
AUC_{0-60} (min × μg/mL)	37.9 ± 2.8	27.9 ± 2.2	29.2 ± 2.4	33.1 ± 2.0
$AUC_{0-\infty}$ (min × μg/mL)	43.3 ± 2.8	29.9 ± 3.2	31.6 ± 3.3	36.9 ± 2.9
CL (mL/min)	11.9 ± 0.86	17.6 ± 1.37*	16.8 ± 1.58*	1 13.8 ± 0.77
Ae_{0-60} (μg)	95 ± 3	234 ± 12*	157 ± 18*	141 ± 15*
$CL_{b,p}$ (mL/min)	2.61 ± 0.25	8.71 ± 0.82*	5.76 ± 0.93*	4.46 ± 0.62*
$CL_{b,l}$ (mL/min)	0.11 ± 0.03	0.18 ± 0.06*	0.11 ± 0.03	0 0.11 ± 0.04
B/L	17.3 ± 1.4	22.4 ± 2.7	15.5 ± 2.3	15.6 ± 1.8
L/P	83 ± 5	266 ± 45*	219 ± 32*	150 ± 20*

*$p < 0.05$ versus control group.

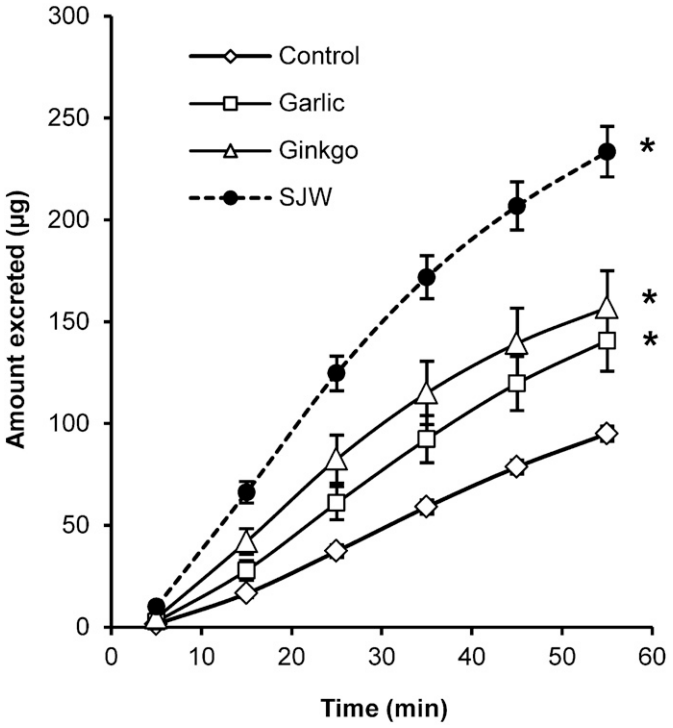

Fig. 4. Influence of garlic (120 mg/kg), ginkgo (17 mg/kg), or SJW (1000 mg/kg) administration on the cumulative biliary excretion of fexofenadine over successive intervals up to 60 minutes. Data points represent the mean ± S.E.M. (n = 7). *$p <$ 0.05 versus control group at 60 minutes.

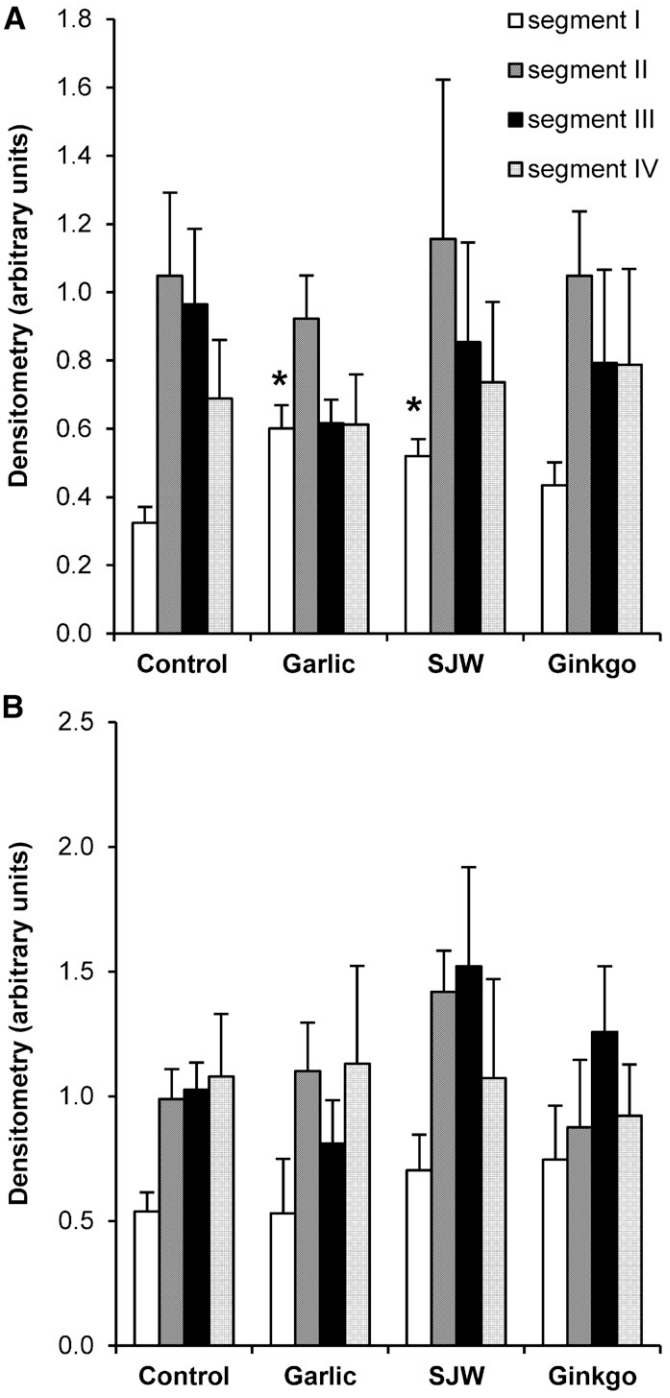

Fig. 5. Effect of 14-day administration of garlic (120 mg/kg), ginkgo (17 mg/kg), and SJW (1000 mg/kg) on the expression of intestinal (A) Oatp1a5 and (B) P-gp. The small intestine was divided into four equal segments, with segment I being the most proximal. Samples were prepared from the intestinal mucosa, as described in *Materials and Methods*. For Oatp1a5 analysis, 100 μg total protein of each segment was loaded onto the gel. For P-gp, 50 μg, 30 μg, 20 μg, and 15 μg were loaded for segments I, II, III, and IV, respectively. Relative expressions were quantified densitometrically and calculated by normalization to the reference bands of β-actin. Data represent mean ± S.E.M. (n = 4–6). *$p <$ 0.05 versus control group.

P-gp (Durr et al., 2000; Kageyama et al., 2006). The expression of P-gp (segment II) in SJW-treated rats was higher than in control rats, but the difference was not significant. Moreover, SJW induced intestinal Oatp1a5 in the present study, but it had no effect on the oral absorption of fexofenadine in vivo. This outcome might also be attributed partly to the increased hepatic CL observed after evaluating the impact of treatment with SJW on hepatic transport in the perfused liver. Ginkgo did not affect the intestinal expression of P-gp or Oatp1a5, and this may explain its lack of effect on the pharmacokinetics and oral absorption of fexofenadine in vivo.

Herbal preparations are standardized to a specific constituent or group of compounds, which may or may not be responsible for the induction of drug-metabolizing enzymes or transporters. SJW preparations are usually standardized to their content of hypericin (Henderson et al., 2002). However, hyperforin is thought to be a more important contributor to any increased metabolism and transport of drugs (Moore et al., 2000; Bauer et al., 2006). Most *Ginkgo biloba* extracts are standardized to 22–27% ginkgo flavonol glycosides and 5–7% terpenoids (Abad et al., 2010). Dried garlic powder tablets are usually standardized to alliin content and/or allicin yield (Amagase et al., 2001; Lawson and Gardner, 2005). A previous study reported that diallyl sulphide increases the expression of CAR target genes in the rat liver (Chang, 2009). Diallyl sulphide is primarily found in garlic oils, and only small amounts are present in tablets (Hajda et al., 2010). Li et al. (2009) ascribed the activation of pregnane X receptor, CAR, and aryl hydrocarbon receptor to terpenoids within ginkgo. However, findings from the effect of such constituents on cellular test systems may not entirely explain the mechanisms of interaction or be applicable in vivo (Venkataramanan et al., 2006). It is unclear which component/s of the garlic and ginkgo is/are responsible for the interactions in the present study.

In conclusion, the results suggest that garlic and ginkgo may induce the activity of hepatic Oatp transport at clinically relevant concentrations. SJW appears to increase both hepatic P-gp and Oatp transport. Garlic and SJW induce the expression of intestinal Oatp1a5. Ginkgo has no effect on

intestinal Oatp1a5 or P-gp. The observations from this study are relevant for any substrate of P-gp and/or Oatp.

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Authorship Contributions

Participated in research design: Turkanovic, Ward, Milne.

Conducted experiments: Turkanovic.

Performed data analysis: Turkanovic, Gerber, Ward, Milne.

Wrote or contributed to the writing of the manuscript: Turkanovic, Ward, Milne.

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