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Research Paper

Effect of St John's wort on the disposition of fexofenadine in the isolated perfused rat liver

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Abstract

Objectives This study examined the effects of St John's wort (*Hypericum perforatum*) on the disposition of fexofenadine, a substrate of P-glycoprotein/organic anion transporting polypeptide, in the isolated perfused rat liver.

Methods Male Sprague-Dawley rats were given St John's wort, 1000 mg/kg, by intragastric gavage once daily for 14 days. On day 15, livers were isolated surgically and perfused in a recirculating system with fexofenadine (2 μg/ml), either alone or following addition of ciclosporin (0.5 μg/ml) 5 min before the addition of fexofenadine. Perfusate samples and bile were collected for 60 min. Fexofenadine in perfusate, bile and the homogenised livers was measured by HPLC.

Key findings Administration of St John's wort significantly increased biliary clearance with respect to perfusate and biliary clearance with respect to the concentration in the liver, by 74% and 71%, respectively. This was reversed by ciclosporin.

Conclusions St John's wort enhanced the elimination of fexofenadine into the bile. This could be because it increases the activity of P-glycoprotein on the canalicular membrane of hepatocytes.

Keywords fexofenadine; hepatic transport; induction; organic anion transporting polypeptide; P-glycoprotein; St John's wort

Introduction

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.[1] Whilst its therapeutic efficacy is comparable to that of tricyclic antidepressants,[2] it has been reported that the extract of SJW alters the disposition of a number of low-therapeutic index drugs – theophylline, digoxin, phenprocoumon and ciclosporin.[3] For example, in transplant patients, co-administration of SJW with the immunosuppressant drug ciclosporin led to a significant decrease in blood levels of ciclosporin and rejection of transplanted organs.[4] It has been suggested that SJW interferes with the transport and metabolism of conventional drugs. Indeed, administration of SJW (1000 mg/kg) to rats for 14 days induced expression of intestinal P-glycoprotein (P-gp) 3.8-fold and hepatic cytochrome P450 (CYP)3A2 by 2.5-fold.[5] Administration of SJW 300 mg three times daily to humans for 14 days caused a decrease (18%) in the oral bioavailability of digoxin, and increased the expression of intestinal P-gp and CYP3A4 by 1.4- and 1.5-fold, respectively. Moreover, a 1.4-fold increase in the activity of hepatic CYP3A4 was observed.[5] Another study reported that the administration of SJW extract (400 mg/kg) to rats for 10 days induced hepatic multidrug resistance-associated protein 2 (MRP2), glutathione S-transferase-P and CYP1A2.[6] Despite this knowledge, a full understanding of the mechanisms of the interactions between SJW and drugs remains incomplete. For instance, the results from studies examining the effect of SJW on the expression of hepatic P-gp are somewhat conflicting. While some studies found no effect of SJW on hepatic P-gp,[5,6] others have concluded that SJW induces its expression and activity.[7,8]

Fexofenadine is an H_1-receptor antagonist that undergoes negligible metabolism in humans and rats.[9,10] Following oral administration of fexofenadine to human volunteers, the majority of the dose was recovered in faeces (80%) and urine (12%) unchanged.[10] Fexofenadine has been identified as a substrate of the efflux transporter P-gp and the influx

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transporter organic anion transporting polypeptide (Oatp).[11] Fexofenadine has therefore been used in a number of studies as a marker substrate to assess the activities of P-gp and/or Oatp.[9,12,13] To date, there are no documented studies that have specifically examined the effect of SJW on the hepatic transport of fexofenadine within the whole organ.

P-gp (also known as ABCB1) is a 170 kDa plasma membrane protein which acts as an ATP-dependent drug efflux transporter. P-gp belongs to the ABC family of membrane transport proteins and is encoded by the multi-drug resistance *MDR1* gene in humans and the *mdr1a/1b* (*Abcb1a/1b*) genes in rodents. It is expressed in a variety of tissues, including the apical border of intestinal epithelial cells, brain capillary endothelial cells, the bile canalicular membrane of hepatocytes and renal proximal tubular epithelial cells.[14] P-gp actively pumps selected drugs/xenobiotics from the intracellular to the extracellular domain. In the liver, P-gp actively secretes drugs and other substances, such as bile acids, across the canalicular membrane into bile.[14–17]

OATPs (SLC21A; Oatps/Slc21a in rodents) are proteins that actively mediate the influx of selected drugs/xenobiotics into cells. They are localized in various tissues, including the apical membrane of intestinal enterocytes and the basolateral membrane of hepatocytes. In the liver, Oatp facilitates the uptake of substrates across the sinusoidal membrane into hepatocytes.[11,18,19] So far, there are no published studies that have investigated the effect of SJW on the expression or activity of Oatp.

Oatp and P-gp have a vast number of substrates, and the importance of their role in the disposition of drugs has been recognized.[14,20] The liver plays an important role in the elimination of drugs and it has been demonstrated that Oatp and P-gp work in tandem within this organ.[11] Therefore, the aim of our study was to investigate the effects of SJW on the hepatic transport of the P-gp/Oatp substrate fexofenadine in the isolated perfused rat liver, in the presence and absence of ciclosporin, a P-gp/Oatp inhibitor.

Materials and Methods

Chemicals

Fexofenadine HCl was donated by Hoechst Marion Roussel, Inc. (Bridgewater, NJ, USA). Ciclosporin was purchased from Sigma Chemical Co. (St Louis, MO, USA). SJW tablets (Kira LI-160 extract, 300 mg, standardised to contain 900 μg hypericin, Lichtwer Pharma AG, Berlin, Germany) were purchased from Thompson's Nutrition (Auckland, NZ). Chemicals for the perfusion experiments were of analytical grade and used as supplied commercially. Acetonitrile (BDH, Poole, England), potassium dihydrogen orthophosphate (AR grade, Ajax Chemicals, Auburn, Australia) and Milli-Q purified water (Millipore, USA) were used for HPLC analysis.

Animals

Male Sprague-Dawley rats (250–350 g) from the Institute of Medical and Veterinary Science (Adelaide, Australia) were housed separately in plastic cages in the animal facility of the University of South Australia under controlled conditions (12 h light–dark cycle) with free access to food (Rat and Mouse Pellets, Specialty Feeds, Glen Forrest, Australia) and tap water at all times. Ethical approval for their use in this study was obtained from the Institute of Medical and Veterinary Science Animal Ethics Committee (Adelaide, Australia).

Experimental design

An SJW suspension was prepared by grinding the tablets, dampening the grounds with ethanol (1.6%) and diluting to the required volume with Milli-Q water. The suspension was protected from light and used within 24 h.

Rats were randomly divided into two control groups and two treatment groups (all $n = 5$). Rats in the treatment groups were dosed orally with SJW (1000 mg/kg) once daily for 14 days; control rats received vehicle only. On day 15, all livers were isolated surgically and perfused as described below.

Liver perfusion

Rats were anaesthetised with 60 mg/kg sodium pentobarbitone (Nembutal, Boehringer Ingelheim, North Ryde, Australia). Livers were perfused *in situ* as described previously.[11] In brief, the livers were perfused via the hepatic portal vein at a flow rate of 30 ml/min in a single-pass manner, at 37°C, with a medium based on Krebs–Henseleit buffer (pH 7.4) for 15 min. During the perfusion, the medium was gassed continuously with a mix of 95% oxygen and 5% carbon dioxide. After allowing time for the liver to equilibrate, drug(s) of interest were added to the recirculation reservoir (250 ml) and the perfusion switched to recirculating mode. Livers from one control group and one treatment group were perfused with fexofenadine HCl at an initial concentration of 2 μg/ml. Livers from the other control and treatment groups were perfused with ciclosporin at an initial concentration of 0.5 μg/ml for 5 min before the addition of fexofenadine.

Perfusate samples (1 ml) were taken from the reservoir at 0, 1, 3, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min after the addition of fexofenadine HCl. All bile was collected over six 10 min periods. Viability of the liver throughout each experiment was assessed as described previously.[11] After each perfusion experiment, perfusate and bile samples and the liver were stored at −20°C pending analysis for fexofenadine.

Measurement of fexofenadine HCl concentration

Concentrations of fexofenadine HCl in the perfusate, bile and liver were measured using an adaptation of previously published HPLC methods.[11,13] The HPLC system consisted of a SIL-10AD auto injector, SPD-10AV UV detector, C-R5A chromatopac integrator (all from Shimadzu Corp., Kyoto, Japan) and 655A-11 pump (Hitachi Corp., Tokyo, Japan). Perfusate and bile samples were thawed, mixed and centrifuged briefly before analysis. Perfusate was analysed undiluted. Bile samples were diluted 1 : 500 with drug-free perfusate. Samples (100 μl) were injected onto a platinum EPS C_{18} 100Å 5 μm (250 mm × 4.6 mm) analytical column protected by a C_{18} precolumn (Alltech, IL, USA). The mobile phase consisted of acetonitrile and 0.024 M potassium dihydrogen orthophosphate

(42 : 58 v/v, pH adjusted to 3.6 using 1 M orthophosphoric acid) delivered at a flow rate of 1 ml/min. Fexofenadine HCl was quantified in the eluent by UV absorbance at 225 nm at a retention time of about 10.4 min. Calibration standards of fexofenadine HCl were prepared using drug-free perfusate. Calibration curves were constructed using linear regression without weighting. The accuracy and precision of the quality control samples spanning the calibration concentrations were within 13%. The lower limit of quantification was 20 ng/ml. The stability of fexofenadine HCl at −20°C for up to 90 days had been demonstrated previously.[11]

Livers were weighed and homogenised in an equal volume of water. Homogenate (0.7 ml) was mixed with an equal volume of acetonitrile, vortexed and centrifuged at 1000*g*. The supernatant was passed through a 0.45 μm syringe filter, and 100 μl injected on to the HPLC column. Calibration standards and quality controls were prepared using blank homogenate. Intra-day accuracy was found to be within 20%.

Pharmacokinetic and statistical analysis

The concentrations of fexofenadine HCl in perfusate up to 60 min were used to calculate the area under the concentration–time curve from 0 to 60 min ($AUC_{0–60}$) and area from 0 to infinity ($AUC_{0–\infty}$); the latter was used to calculate the clearance (CL) of fexofenadine HCl from the perfusate using WinNonlin (Professional version 4.0: Pharsight Corp., Mountain View, CA, USA). The cumulative amount of fexofenadine HCl excreted into bile from time 0 to 60 min ($Ae_{0–60}$) was the summed products of the biliary volume and concentration of fexofenadine HCl during each collection interval up to 60 min. The biliary clearance with respect to perfusate ($CL_{b,p}$) was the quotient of $Ae_{0–60}$ and $AUC_{0–60}$. The biliary clearance with respect to the concentration in liver ($CL_{b,l}$) was the quotient of the rate of excretion of fexofenadine HCl into bile at the 50–60 min collection interval and its concentration in the liver at 60 min. The ratios of the concentrations for liver to perfusate (L/P) and bile to liver (B/L) were calculated using the respective concentrations at 60 min.

All data were tested for normality and homogeneity of variance (using SPSS 15.0 for Windows, Chicago, IL, USA). In cases where the normality test failed, data were log-transformed before statistical analysis. Single-factor analysis of variance was used to test for differences between the experimental groups. For AUC, $Ae_{0–60}$, CL, $CL_{b,p}$, $CL_{b,l}$ and B/L, post-hoc comparisons were conducted using the method of least significant difference. L/P ratios were compared using the Dunnett's multiple comparisons test. Differences between groups were considered statistically significant at $P < 0.05$. All values are presented as arithmetic mean ± SEM.

Results

Livers were perfused evenly throughout all perfusion experiments. Bile flow was 5–10 μl/min, with no significant differences between the groups. The pH of the inflow and outflow perfusion medium was 7.35–7.45 and 7.20–7.35, respectively.

Figure 1 shows concentration–time profiles of fexofenadine HCl expressed as a percentage of the initial concentration in perfusate for the four groups of rats. The concentration of fexofenadine HCl in the perfusate decreased with time as fexofenadine HCl was cleared from the perfusate by the liver and excreted into bile. From Figure 1 it is evident that the concentration of fexofenadine HCl in the perfusate declined more slowly during concurrent perfusion with ciclosporin. Figure 2 shows the influence of SJW and ciclosporin on the cumulative excretion of fexofenadine HCl into bile. Administration of SJW to rats caused a significant increase of 61% in the $Ae_{0–60}$ of fexofenadine. In the presence of ciclosporin, the $Ae_{0–60}$ decreased significantly in both the control (44%) and SJW-treated (50%) groups. The $CL_{b,p}$ in the SJW-treated group was increased significantly ($p < 0.05$) by 74% over the control group (Figure 3). Conversely, the $CL_{b,p}$ was decreased significantly ($p < 0.05$) by ciclosporin in both the control (66%) and SJW-treated (69%) rats (Figure 3). There were no differences in CL between the control (21.8 ± 2.5 ml/min) and SJW-treated (25.5 ± 2.6 ml/min) groups (Figure 3). However, CL decreased significantly in the presence of ciclosporin in both the control (43%) and SJW-treated (46%) groups (Figure 3).

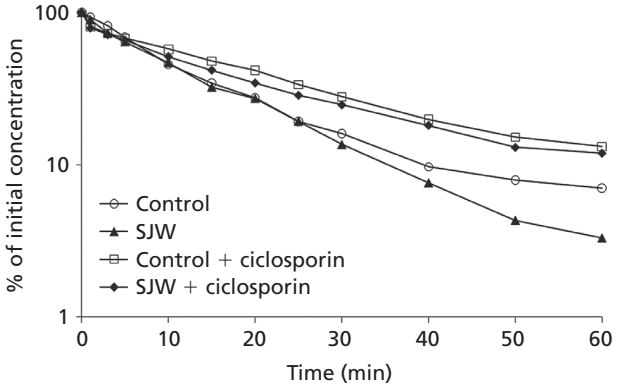

Figure 1 Concentration–time profiles of fexofenadine HCl in perfusate. Livers from rats treated previously with St John's Wort (SJW; 1000 mg/kg for 14 days) were perfused with fexofenadine HCl (2 μg/ml) in the presence and absence of ciclosporin (0.5 μg/ml). Data points represents mean of 5 rats per group. Error bars have been omitted for clarity.

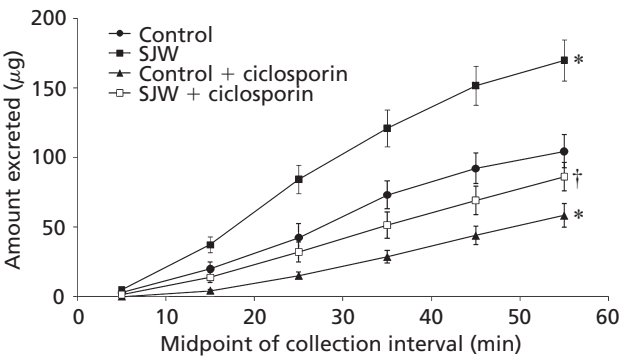

Figure 2 Influence of St John's wort and ciclosporin on the cumulative biliary excretion of fexofenadine HCl. Data points represent means ± SEM ($n = 5$). *$P < 0.05$ vs control group at 60 min; †$P < 0.05$ vs St John's wort (SJW) group at 60 min.

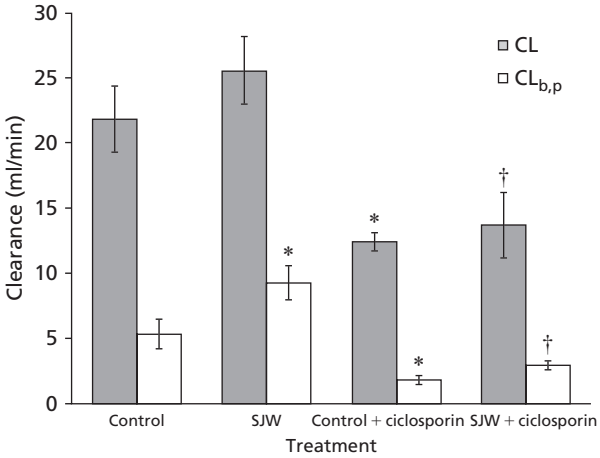

Figure 3 Clearance of fexofenadine HCl from the perfusate. Fexofenadine HCl clearance (CL) is compared with the biliary clearance with respect to perfusate ($CL_{b,p}$) in perfused livers from rats treated with St John's wort (SJW; 1000 mg/kg for 14 days). Bars show means ± SEM ($n = 5$). *$P < 0.05$ vs control group; †$P < 0.05$ vs SJW group.

The $CL_{b,l}$ in the SJW-treated group was increased significantly ($p < 0.05$) by 71% compared with the control group (Table 1). In the presence of ciclosporin, the $CL_{b,l}$ decreased significantly ($p < 0.05$) in the SJW-treated group (42%) but did not change in the control group (Table 1). There were no significant differences in the ratios of B/L and L/P between the control and SJW-treated groups (Table 1). During concurrent perfusion with ciclosporin, the B/L ratio decreased in the SJW-treated group ($p < 0.05$), whereas the L/P ratio was decreased by ciclosporin in both the control and SJW-treated groups ($p < 0.05$).

Discussion

The Kira LI-160 extract used in our study is the most extensively researched SJW preparation worldwide and was used in the majority of European clinical trials investigating the therapeutic efficacy of SJW.[21] This SJW preparation, like many others, is available as an over-the-counter medicine in most countries. SJW is often taken without the knowledge of health professionals and administered concomitantly with conventional drugs. This has the potential to cause fatal adverse reactions.[22] Therefore, a full understanding of its possible mechanisms of interaction with other drugs is important so that the potential impact on oral availability and clearance of the absorbed drug is fully appreciated.

It is well established that SJW induces cytochrome P450 enzymes, intestinal P-gp and various other transporters in humans and rats.[5,6,23] Studies investigating the effect of SJW on the expression of hepatic P-gp have produced different results however.[5–8] In contrast, there are no published studies that have investigated the effect of SJW on the expression or activity of Oatp. Therefore, the present study was designed to investigate the potential for SJW to affect the hepatic transport of fexofenadine, a probe substrate for P-gp/Oapt[11] in the isolated perfused rat liver. The dose and the duration of SJW treatment were chosen on the basis of previous reports.[5,8] Considering that the metabolism of fexofenadine is minor,[10] and that the uptake and excretion of fexofenadine in rats is mediated by hepatic Oatp and P-gp, respectively,[11] any alterations in the pharmacokinetics of fexofenadine in the current study can be correlated directly to changes in the activity of hepatic P-gp and/or Oatp.

Pretreatment of rats with SJW 1000 mg/kg significantly increased $CL_{b,p}$ (Figure 3) and $CL_{b,l}$ (Table 1) of fexofenadine, by 74% and 71%, respectively. This demonstrates that SJW alters the hepatic disposition of fexofenadine and enhances its elimination into bile. Previous studies[11,13] have shown that B/L and L/P ratios can be used to determine whether P-gp and/or Oatp are responsible for changes in the $CL_{b,p}$ of fexofenadine. According to that model, a significant increase in L/P but no change in B/L indicates that Oatp is induced. Conversely, a significant increase in B/L with no change in L/P indicates induction of P-gp. In our study, the ratios could not be used to determine whether the increase in $CL_{b,p}$ was due to enhanced sinusoidal or canalicular transport, as their values did not change significantly with SJW treatment. This could be due to the high variability between animals, which is reflected in the high values of standard error for the ratios in all groups of rats. However, since the $CL_{b,l}$, which approximated the effect of SJW on the excretion of fexofenadine across the canalicular membrane relative to concentrations within liver tissue, was found to be significantly increased by SJW, we believe that the enhanced biliary elimination of fexofenadine is due to upregulation of canalicular transport.

While the results indicate that canalicular transport of fexofenadine was induced by SJW, the effect on overall CL was equivocal (Figure 3). It might be expected that induction of excretion across the canalicular membrane would lead to a decline in the concentrations of fexofenadine in the

Table 1 Influence of St John's wort (1000 mg/kg for 14 days) and ciclosporin (0.5 μg/ml) on the pharmacokinetic parameters of fexofenadine HCl in the isolated perfused rat liver

Parameter	Control	SJW	Control + ciclosporin	SJW + ciclosporin
$CL_{b,l}$ (ml/min)	0.14 ± 0.01	0.24 ± 0.02*	0.13 ± 0.02	0.14 ± 0.04†
B/L	24.9 ± 5.5	32.2 ± 2.0	18.7 ± 2.8	21.5 ± 8.6†
L/P	133 ± 35	194 ± 21	56 ± 3.6*	95 ± 29†

Values are means ± SEM ($n = 5$ rats). $CL_{b,l}$, biliary clearance with respect to the concentration in the liver; B/L, ratio of concentrations of fexofenadine in bile to liver; L/P, ratio of concentrations of fexofenadine HCl in liver to perfusate. *$P < 0.05$ vs control, †$P < 0.05$ vs SJW-treated group.

perfusate, as more fexofenadine would be excreted into the bile. A similar observation to that in the present study occurred in a previous study investigating the effect of erythromycin and dibromosulphothalein on the disposition of fexofenadine in the isolated perfused rat liver.[11] In that study, there were no significant alterations in CL despite substantial changes in the $CL_{b,p}$ of fexofenadine. Since metabolism is a minor route for the elimination of fexofenadine, the only anticipated route of removal is via biliary excretion. However, there was clearly a delay in its transfer from perfusate to bile. For example, concentrations in perfusate decreased by approximately 80% between 20 and 30 min whereas less than 25% of the overall amount excreted in bile was recovered in bile at the end of this interval. Therefore, a large fraction of fexofenadine may have been taken up by the liver and retained within the hepatocytes, meaning that the change in $CL_{b,p}$ over the duration of the perfusion experiment was not sufficient to have an impact on overall CL which, in this instance, represents both biliary clearance and uptake from perfusate into hepatocytes.

The inhibitory effect of ciclosporin on P-gp and Oatp has been demonstrated previously.[24] In the present study, ciclosporin was used to determine whether any potential increases in the hepatic transport of fexofenadine by SJW would be reversed. Preliminary experiments in our laboratory had shown that addition of a single dose of ciclosporin into the perfusate, generating a nominal concentration of 0.5 μg/ml, inhibited the hepatic transport of fexofenadine without causing liver toxicity. Concurrent perfusion with ciclosporin decreased CL, $CL_{b,p}$ and L/P significantly in both the control and SJW-treated groups. A decrease in L/P suggests that ciclosporin inhibited fexofenadine uptake via Oatp. Interestingly, B/L and $CL_{b,l}$ decreased in the SJW-treated group only. This suggests either that other canalicular transporters compensate when P-gp is inhibited by ciclosporin or that canalicular transporter(s) other than P-gp were induced and contributed to increased biliary clearance of fexofenadine. Various other drug transporters are located in the bile canalicular membrane of the hepatocytes. These include MRP2 (ABCC2), breast cancer resistance protein (BCRP/ABCG2) and the bile salt export pump (BSEP/ABCB11).[25] MRP2 was found to play a role, albeit a limited one, in the biliary excretion of fexofenadine in rats,[26] and preliminary data suggest that BSEP may have a similar role.[27] It is possible that the contribution of transporters that play limited roles in the disposition of a particular drug under normal circumstances are increased by treatment with inducers. Indeed, SJW has been shown to be a potent inducer of hepatic MRP2 expression.[6] In addition, studies have identified ciclosporin as an MRP2 inhibitor.[28] Thus, we cannot exclude the possibility that MRP2 and/or an as-yet unidentified transport mechanism contributed to the increased biliary clearance of fexofenadine following treatment with SJW in our study. The overall results from the present study are summarised in Figure 4.

Many different SJW preparations are available, and commercial SJW preparations contain over 20 constituents that contribute to its pharmacological effect.[29] The main active constituents of SJW are thought to be hypericin, hyperforin and flavonoids.[30] SJW products are generally

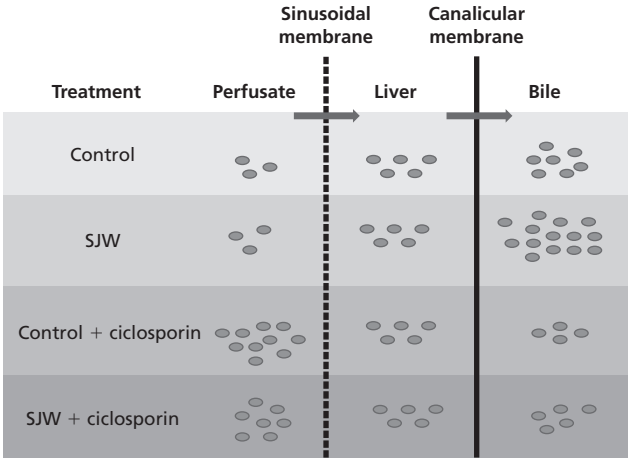

Figure 4 Summary of the results obtained in the present study. Fexofenadine HCl is actively transported across the sinusoidal membrane into the liver and is then excreted across the canalicular membrane into bile. St John's wort (SJW) increased the excretion of fexofenadine across the canalicular membrane. Ciclosporin decreased the transport of fexofenadine from the perfusate into liver and inhibited the transport from liver into bile. The SJW-induced transport across the canalicular membrane is reversed by ciclosporin.

standardised to a certain content of hypericin.[4] However, hyperforin is thought to be responsible for the increased metabolism and/or transport of various P-gp and cytochrome P450 substrates.[29,31] For instance, a recent study has demonstrated that SJW extracts with a low hyperforin content, or none at all, did not cause any significant changes to the pharmacokinetics of the P-gp substrate digoxin.[32] In contrast, the same study showed that the LI-160 extract, which was determined to be richer in hyperforin, significantly reduced the area under the curve for digoxin.[32] Thus, the observed interaction in the current study could be attributed to the high content of hyperforin in the LI-160 extract, and the interaction may not necessarily occur with all SJW preparations.

Conclusions

SJW pretreatment causes a substantial increase in the biliary clearance of fexofenadine, which may be due to increased excretion across the canalicular membrane. This observation is consistent with an up-regulation of hepatic P-gp transport. There is no evidence from our data for any impact of SJW on the transport of fexofenadine across the sinusoidal membrane. The results from this work give a greater insight into the mechanisms underlying interactions between SJW and conventional drugs and have significant implications for drugs that are cleared from the body via hepatic transport.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

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