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Saint John's wort: An *in vitro* analysis of P-glycoprotein induction due to extended exposure

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1 Chronic use of Saint John's wort (SJW) has been shown to lower the bioavailability for a variety of co-administered drugs including indinavir, cyclosporin, and digoxin. Decreases in intestinal absorption through induction of the multidrug resistance transporter, P-glycoprotein (P-gp), may explain decreased bioavailability.

2 The present study characterized the response of P-gp to chronic and acute exposure of SJW and hypericin (HYP, a presumed active moiety within SJW) in an *in vitro* system. Experiments were performed with 3 to 300 μg ml^{-1} of methanol-extracted SJW and 0.03 to 3 μM HYP, representing low to high estimates of intestinal concentrations.

3 In induction experiments, LS-180 intestinal carcinoma cells were exposed for 3 days to SJW, HYP, vehicle or a positive control (ritonavir). P-gp was quantified using Western blot analysis. P-gp expression was strongly induced by SJW (400% increase at 300 μg ml^{-1}) and by HYP (700% at 3 μM) in a dose-dependent fashion. Cells chronically treated with SJW had decreased accumulation of rhodamine 123, a P-gp substrate, that was reversed with acute verapamil, a P-gp inhibitor. Fluorescence microscopy of intact cells validated these findings. In Caco-2 cell monolayers, SJW and HYP caused moderate inhibition of P-gp-attributed transport at the maximum concentrations tested.

4 SJW and HYP significantly induced P-gp expression at low, clinically relevant concentrations. Similar effects occurring *in vivo* may explain the decreased bioavailability of P-gp substrate drugs when co-administered with SJW.

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Abbreviations: CYP3A, cytochrome P450 3A; DMSO, dimethyl sulphoxide; HPLC, high performance liquid chromotography; HYP, hypericin; P-gp, P-glycoprotein; SJW, Saint John's wort

Introduction

Saint John's wort (SJW) is a flower extract from *Hypericum perforatum*, used for centuries in holistic medicine to accelerate wound healing and treat nerve pain. In recent years, SJW has been recognized as an effective antidepressant both anecdotically and in placebo-controlled clinical trials (Brenner *et al*., 2000; Linde *et al*., 1996; Schrader, 2000; Woelk, 2000). Throughout Europe and in the United States, millions of SJW doses are taken daily, whether by doctor's prescription or 'over-the-counter' self medication (Canedy, 1998; Lohse & Müller-Oerlinghausen, 1998). With increasingly prevalent use, interactions of SJW with co-administered medications have been reported. Chronic use of SJW reduces the bioavailability of a number of drugs, including the HIV protease inhibitor indinavir, the immunosuppressant cyclosporin, and the cardiac glycoside digoxin (Johne *et al*., 1999; Piscitelli *et al*., 2000; Ruschitzka *et al*., 2000). Co-administration of SJW decreased indinavir AUC by 57% and estimated plasma trough values by 81% in healthy volunteers (Piscitelli *et al*., 2000). Acute heart transplant rejection due to decreased cyclosporin plasma levels (greater than 50% reduction) with co-administration of SJW has been reported in several case studies (Ruschitzka *et al*., 2000), and similar findings have been seen in kidney transplant patients (Breidenbach *et al*., 2000a; Mai *et al*., 2000).

The SJW drug interaction, in which the bioavailability of orally dosed drugs is lowered, could be attributed to cytochrome P450 3A (CYP3A) and/or P-glycoprotein (P-gp) induction. CYP3A isoforms are the major phase I metabolizing enzymes found in human liver, kidney, and small intestine (Watkins, 1997) and metabolize more than 50% of drugs used in clinical practice (Benet *et al*., 1996). P-gp is an ATP-dependent drug transporter, expressed in the epithelia of the kidney proximal tubules, the small and large intestine, and the biliary hepatocytes where it plays a role in drug clearance and metabolism (Gottesman *et al*., 1996). P-gp is also present in capillary endothelial cells of the brain, testis, adrenal gland, and placental trophoblasts where it serves a barrier function (Choo *et al*., 2000; Schinkel *et al*., 1994). It is here that P-gp can reduce toxin accumulation or have the potential to create drug sanctuaries in disease therapies such as that of HIV and leukaemia. Most P-gp transport substrates are also substrates for CYP3A mediated metabolism (Gottesman *et al*., 1996; Wacher *et al*., 1995), however the reverse is not necessarily true.

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The concurrent action of intestinal CYP3A and P-gp creates an absorption barrier that influences the oral bioavailability of many medications (Wacher *et al*., 1996; Watkins, 1997).

Several studies in rats and humans have previously indicated the potential for CYP3A and P-gp induction due to SJW exposure (Durr *et al*., 2000; Johne *et al*., 1999; Roby *et al*., 2000; Westphal *et al*., 2000). SJW caused a 3.8 fold and 2.5 fold increase in intestinal P-gp and CYP3A, respectively, after 14 days chronic administration in rats. Similarly in human studies, an approximate 1.5 fold increase of both proteins was seen with intestinal biopsies following chronic administration of SJW (Durr *et al*., 2000). Identifying and separating the relative contributions of induction of CYP3A and P-gp activity due to SJW is difficult considering most drugs affected are substrates for both. Increased generation of the CYP3A specific metabolite 6-β-hydroxycortisol in the urine of human volunteers after 14 days of SJW treatment suggests increased CYP3A activity (Roby *et al*., 2000). However, this methodology does not elucidate any organ clearance specificity, whether liver, intestine, or kidney. The route of enhanced clearance (or decreased absorption) appears paramount in understanding the mechanism of the SJW interaction, since only adverse interactions with orally administered drugs have been reported. In animal studies, where dexamethasone induced both P-gp and CYP3A, clearance of IV indinavir was not significantly affected (Lin *et al*., 1999). However, orally administered indinavir had a 10 fold decrement in the C_{max} and a 3 fold reduction in AUC (Lin *et al*., 1999). The previous studies taken collectively suggest decrements in bioavailability from chronic SJW are taking place predominately in the intestine.

While many CYP3A metabolized drugs are given orally, only those that are also substrates for P-gp transport have thus far been reported to have clinically significant drug interactions with SJW. In addition, drugs that are not CYP3A metabolized can still be affected by SJW. Digoxin is a P-gp substrate (Greiner *et al*., 1999), that is largely unmetabolized in humans (Lacarelle *et al*., 1991). After 10 days of SJW treatment, oral digoxin had a 25% reduction of plasma AUC and C_{max} (Johne *et al*., 1999). Both intestinal P-gp and CYP3A can be induced by SJW (Durr *et al*., 2000) and while extensive *ex vivo* and *in vitro* studies have been done to understand (and separate) CYP3A expression and activity in the SJW drug interaction (Durr *et al*., 2000; Moore *et al*., 2000; Obach, 2000; Wentworth *et al*., 2000), such *in vitro* examination has not been done for P-gp. *In vitro* examination of P-gp expression and mediated activity is necessary to further explain the mechanism of the SJW drug interaction, separate its activity from that of CYP3A, and predict potential P-gp attributed effects *in vivo*. The following study was designed to specifically examine P-gp induction *in vitro* as a potential mechanism to explain the SJW drug interaction, and to evaluate this model as an approach to predicting clinical drug interactions due to P-gp induction.

Methods

Chemicals and antibodies

St. John's wort (Natrol Inc., Chatsworth, CA, U.S.A.) and ritonavir were extracted into methanol solution from commercially available dosage forms. Purity was verified by HPLC against pure hypericin (an active moiety within SJW, Carl Roth GmbH & Co., Karlsruhe, Germany) and ritonavir standard (Abbott Laboratories, N. Chicago, IL, U.S.A.). Solvent was removed before use in cell cultures. Rhodamine 123, verapamil, quinidine, probenicid, indomethacin, vinblastine and dimethyl sulphoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). C219 monoclonal antibodies to human P-gp were purchased from Signet Co. (Dedham, MA, U.S.A.).

Cell lines and growth conditions

The human colon adenocarcinoma cell line, LS-180 (Tom *et al*., 1976), was obtained from ATCC (Manassas, VA, U.S.A.) and has been used previously to study P-gp induction (Perloff *et al*., 2000; Schuetz *et al*., 1996; Störmer *et al*., 2001). LS-180 cell media consisted of minimal essential media and Earl's salts (GibcoBRL, Grand Island, NY, U.S.A.) supplemented with 10% foetal bovine serum, 0.1 mM non-essential amino acid solution (Gibco-BRL), 1 mM pyruvic acid, 100 units ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin. The selected cell line, LS-180V (passages 30–50), was maintained with 4 ng ml^{-1} vinblastine as previously described (Herzog *et al*., 1993; Perloff *et al*., 2000) until the initiation of induction and drug accumulation experiments. The human colon adenocarcinoma cell line Caco-2 (passages 30–40) was kindly provided by Douglas Jefferson, Ph.D. (Tufts University School of Medicine and the New England Medical Center, Boston, MA, U.S.A.). Caco-2 cells are commonly used to study P-gp transepithelial activity (Alsenz *et al*., 1998; Cavet *et al*., 1996; Yumoto *et al*., 1999). Caco-2 cells were grown in DMEM (Dulbecco's Modified Eagle's Medium, Gibco-BRL) supplemented with 10% foetal bovine serum, 0.1 mM non-essential amino acids (Gibco-BRL), 100 units ml^{-1} penicillin, and 0.1 mg ml^{-1} streptomycin. All cells were incubated at 37°C in a humidified chamber with 5% carbon dioxide, with a media change every 3–4 days.

P-gp induction

LS-180V cells were plated in 50 mm culture dishes or six wells plates and grown to a density of 50% confluence. DMSO, 0.5%, was included in the media to ensure drug dissolution and cells were incubated with SJW (3, 10, 30, 100, 300 μg ml^{-1}), hypericin (HYP) (0.03, 0.1, 0.3, 1, 3 μM) or 10 μM ritonavir (positive control) and 0.5% DMSO alone for 72 h ($n=3$). Cells were then collected for Western blot analysis or washed five times with warm ($\sim$37°C) media proceeding rhodamine 123 accumulation studies.

Western blotting

Western blot procedures were done as previously described (Perloff *et al*., 2000). Briefly, cells were collected, lysed with 1% triton-X and 0.5% deoxycholate, (in phosphate buffer, pH 7.4 with 1 mM phenylmethylsulphonyl fluoride), spun down ($1000 \times g$) and the supernatant was collected. The total protein concentration was determined by a bicinchoninic acid protein assay (Pierce, Rockford, IL, U.S.A.) with bovine serum albumin as a standard. Sample protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis on a 4–15% gradient polyacrylamide gel and immunoblotted

using monoclonal mouse anti-human MDR1 (1 : 1000, C219) with sheep anti-mouse Ig-horseradish peroxidase (1 : 3000) as the secondary antibody. SuperSignal West Pico Chemiluminescent Substrate (Pierce) was used to activate the horseradish peroxidase signal. Blots were then exposed to radiographic film and the quantification of protein was completed *via* computer image analysis (Scion Image, Scion Corp., Frederic, MD, U.S.A.), band integrated density was determined based on comparison with a standard curve. Highly induced/concentrated LS-180V cell preparations were used to generate a relative standard curve at various known total protein concentrations (linear band signal up to 25 μg) (Figure 1). The calibration curve allowed for comparisons of relative P-gp contents between samples. Determination of absolute P-gp content requires a pure P-gp standard that is currently not available. Intrablot variability (with blot error) was 7.1%. Interday variability (between day, different blots) was 17.4%.

Drug accumulation studies

LS-180V cells were preincubated for 30 min with 100 μM verapamil, 100 μM quinidine, 100 μM ritonavir, 100 μM indomethacin, 2 mM probenicid, 3 μM HYP, 300 μg ml^{-1} SJW, or media (0.5% DMSO) alone. Rhodamine 123 solution was then added to each well such that they contained 10 μM rhodamine 123 and 1% methanol. After incubation for 60 min, the cells were thoroughly washed five times with warm media. Cells were then solubilized with 0.5% deoxycholate and 1% triton X. The supernatant from each well was then analysed for rhodamine 123 fluorescence and total cell protein.

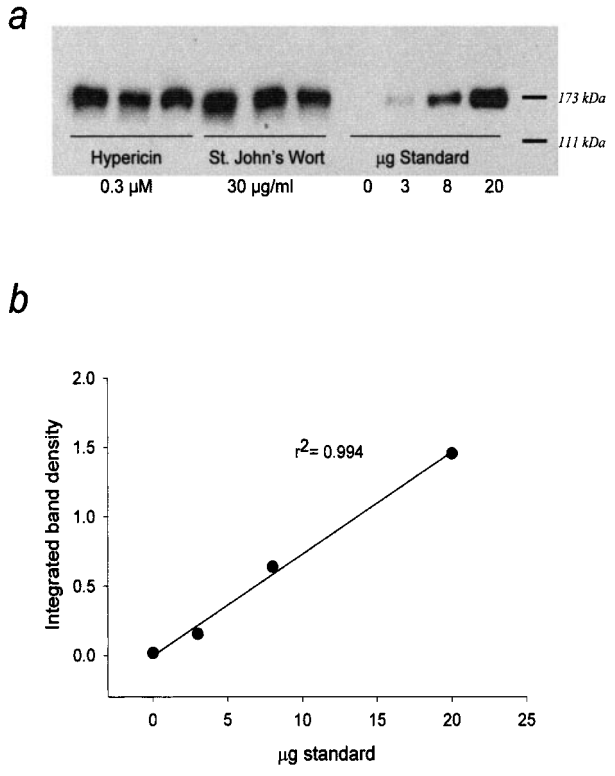

Figure 1 Quantification of P-gp Western blots. (a) Representative Western blot of HYP (0.3 μM) and SJW (30 μg ml^{-1}) treated samples with a standard curve (0, 3, 8, and 20 μg standard). (b) Standard curve generated *via* computer image analysis (integrated band density, Scion Image, Scion Corp.) used to quantify immunoblot samples. The standard curve was consistently linear up to 25 μg standard, all samples were diluted to be within this range.

Fluorescence microscopy

Drug accumulation experiments were performed as described above but the cells were not lysed. Minutes after the final wash, LS-180V cell monolayers were analysed by epifluorescence microscopy using a Nikon Optishot microscope. Rhodamine 123 was visualized using an FITC filter set (ex: 450–490, em: 510–530). Images were captured using a Photometric Quantix Digital Camera and V++ software (Digital Optics, Auckland, New Zealand). Incandescent images of the same field were also taken to demonstrate similar cell density in the various conditions.

Inhibition of P-gp mediated transport in Caco-2 cell monolayers

Caco-2 cells were seeded at 2×10^4 (cm^2)$^{-1}$ in polycarbonate membrane transwell plates (3 μm pore size) (Corning Costar Corp., Cambridge, MA, U.S.A.) and experiments were conducted on days 18–24 post seeding. Drug solutions were prepared in Opti-MEM (Gibco-BRL) serum free media containing 0.5% DMSO and 0.5% methanol. Opti-MEM media containing 5 μM rhodamine 123 was added to the apical or basolateral chamber. Additional plates with SJW (3, 10, 30, 100, 300 μg ml^{-1}), HYP (0.03, 0.1, 0.3, 1, 3 μM), ritonavir (1, 3, 10, 25, 100 μM) or verapamil (100 μM) present in both chambers were tested to ascertain if rhodamine 123 transport was impaired. After 120 min incubation, aliquots were removed from the chamber initially not containing rhodamine 123 and subjected to fluorescence analysis.

Rhodamine 123 fluorometric analysis

Rhodamine 123 was quantified with fluorometric analysis, 500 nm (excitation) and 550 nm (emission), using a Perkin Elmer LS50B luminescence spectrophotometer. Picomoles of rhodamine 123 in samples were determined based on calibration curves constructed from a series of standards.

Data analysis

Drug accumulation values are presented as the mean of three individual trials ($\pm$standard deviation) using three sister culture plates ($n=3$). Comparison among groups to their respective vehicle controls were performed using Kruskal-Wallis ANOVA on ranks with Student-Newman-Keuls analysis.

Results

Induction of P-gp protein by SJW and HYP

Both SJW and HYP caused a concentration-dependent induction of P-gp protein in the LS-180V intestinal cells compared to the DMSO vehicle controls (Figure 2). Western blot quantification ($n=3$) demonstrated significant P-gp

induction. SJW caused a 2 fold induction at 30 μg ml^{-1} and a 4 fold induction at 300 μg ml^{-1}. HYP caused a 3 fold induction at 1 μM and a 7 fold induction at 3 μM. Ritonavir (10 μM), the positive control for P-gp induction, had an expected inductive effect on P-gp protein expression ($\sim$4 fold, data not shown). This effect is consistent with previous studies (Perloff *et al*., 2000).

Intracellular accumulation of rhodamine 123

In acute exposure inhibition experiments, only the known P-gp inhibitors (ritonavir, quinidine, and verapamil) raised intracellular rhodamine 123 accumulation (>3 fold), while multidrug resistance-associated protein (MRP1) inhibitors (probenicid and indomethacin) and HYP had no effect (Figure 3). SJW caused modest increases in rhodamine 123 cell accumulation at 300 μg ml^{-1}. Chronic SJW exposure (72 h) caused a concentration-dependent decrease in cell accumulation of rhodamine 123 in LS-180V intestinal cells compared to the DMSO vehicle controls (Table 1). The cell accumulation model is limited by the fact that basal levels of rhodamine 123, although not intracellular, cannot be washed out, limiting the apparent maximal effect on cell exclusion. In any case, SJW treatment caused concentration-dependent decreases in rhodamine 123 cell accumulation ($P<0.05$) which correlated well (Spearman rank order correlation, $r^2=0.92$, $P=0.0167$) with increases in P-gp immunoreactive protein. The cell accumulation of rhodamine 123 was decreased more than 50% when cells were treated with 100 μg ml^{-1} SJW. Differences in cell accumulation of rhodamine 123 could be eliminated with 100 μM verapamil (Table 1), a P-gp inhibitor. However, there was still a trend towards less rhodamine 123 in cells treated with higher concentrations of SJW. HYP concentrations greater than 0.1 μM (for 72 h) caused LS-180V cell permeability, greatly elevating intracellular rhodamine 123 concentrations that were unaffected by acute 100 μM verapamil, making cell accumulation as a predictive measure of P-gp activity invalid. Cell accumulation of rhodamine 123 with HYP concentrations of 0.03 and 0.1 μM did not significantly differ from controls.

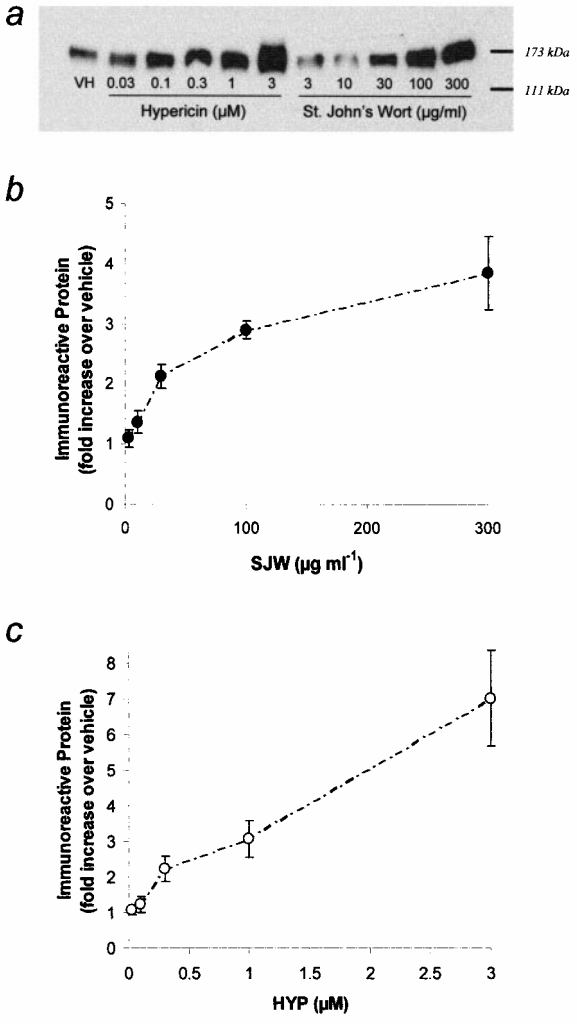

Figure 2 Concentration-dependent induction of P-gp protein by SJW and HYP. LS-180V cells were incubated with vehicle alone (VH, 0.5% DMSO), or SJW (3, 10, 30, 100, 300 μg ml^{-1}) or HYP (0.03, 0.1, 0.3, 1, 3 μM) (a) Qualitative Western blot of immunoreactive P-gp protein demonstrating a concentration-response with both SJW and HYP. (b) and (c) Quantitative Western blotting ($n=3$), fold immunoreactive protein increase compared to vehicle. Blots were quantified *via* computer integrated density analysis with comparison to a standard curve.

Fluorescence microscopy of rhodamine 123 cell accumulation

Epi-fluorescence and incandescence microscopy images of LS-180V cells were taken after 60 min exposure to 10 μM rhodamine 123. Cells with previous chronic exposure (72 h) to 100 μg ml^{-1} SJW or 0.5% DMSO vehicle alone and then incubated with and without acute addition of 100 μM verapamil, were subjected to fluorescence microscopy (Figure 4). The 0.5% DMSO pretreated cells showed accumulation of rhodamine 123 in the cytoplasm and modest concentration in the organelles, most likely the mitochondria (Figure 4a). SJW pretreated cells showed little diffuse rhodamine 123 accumulation (Figure 4b). When both cell populations were subjected to rhodamine 123 exposure in the presence of acute verapamil, they became indistinguishable. With acute exposure to 100 μM verapamil, rhodamine 123 intracellular concentration is increased greatly, with intense fluorescence signal throughout the cell but concentrated in the organelles and cytoplasm (Figure 4c,d). Incandescent images demonstrate similar cell density in each field (Figure 4e–h). However, because rhodamine 123 absorbs incandescent light, the SJW treated cells that resist rhodamine 123 accumulation appear transparent (Figure 4f).

Inhibition of P-gp mediated transport in Caco-2 cell monolayers

The inhibitory potential of acute SJW and HYP on rhodamine 123 transport was tested in conventional Caco-2 monolayers (Figure 5). SJW and HYP caused little inhibition when compared to known P-gp inhibitors like ritonavir or verapamil. SJW and HYP only achieved 40% inhibition of rhodamine 123 transport at the maximum concentrations tested (300 μg ml^{-1} and 3 μM, respectively). Verapamil and ritonavir (100 μM) both achieved potent inhibition of rhodamine 123 transport (>90%). Ritonavir inhibited rhodamine 123 transport with an IC_{50} of 6.64 μM. Consider-

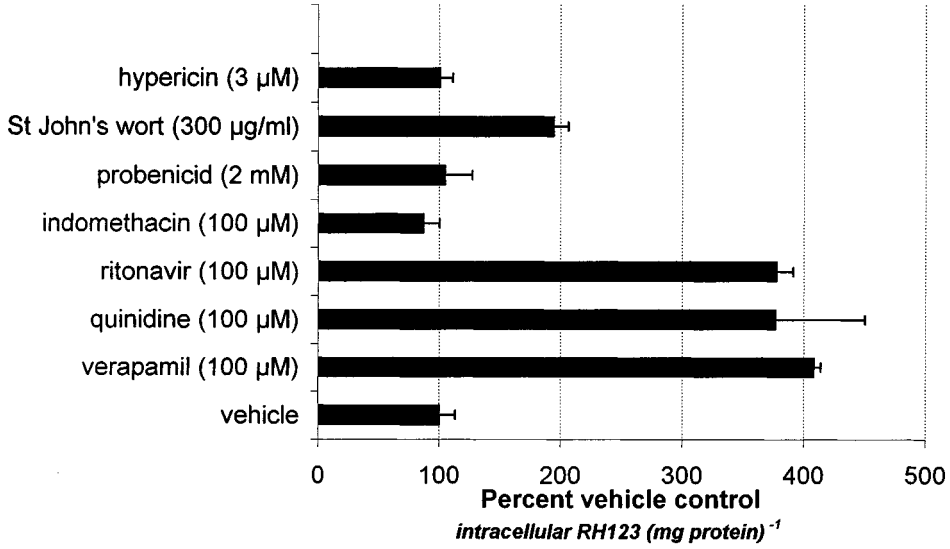

Figure 3 Inhibition of rhodamine 123 efflux. Rhodamine 123 efflux activity from LS-180V cells was determined in presence of known P-gp and MRP1 inhibitors, SJW and HYP. The graph shows intracellular accumulation of rhodamine 123 in the presence of vehicle alone, known P-gp (ritonavir, quinidine, and verapamil) and MRP1 (probenicid and indomethacin) inhibitors, SJW and HYP, ($n=3$) correcting for total cell protein. P-gp inhibitors caused more than a 3 fold increase in rhodamine 123 intracellular concentration, while MRP1 inhibitors and HYP had no effect compared to control values. SJW caused a modest increase in rhodamine 123 intracellular concentration.

Table 1 Cellular accumulation of rhodamine 123 after extended (72 h) exposure to St John's wort

	Media only	*Media with 100 μM verapamil*
Vehicle		
0.5% DMSO	172.6±8.2	733.1±24.2
Ritonavir		
10 μM	86.6±5.4*	587.2±69.6
Saint John's wort		
3 μg ml^{-1}	168.8±17.0	782.2±77.8
10 μg ml^{-1}	121.0±15.8	668.8±16.1
30 μg ml^{-1}	93.2±13.2*	633.6±56.5
100 μg ml^{-1}	78.8±5.2*	549.6±54.9

Values are pmoles per mg protein and are presented as the mean ($n=3$)±s.d. Significant difference from the corresponding DMSO control are indicated by * ($P<0.05$) as determined by Kruskal-Wallis ANOVA on ranks with Student-Newman-Keuls analysis.

ing likely *in vivo* intestinal concentrations (30–100 μg ml^{-1} SJW and 0.3 to 1 μM HYP), the inhibition of transport caused by SJW and HYP is considered minimal.

Discussion

The SJW drug interaction is established to cause a significant decrement in the oral bioavailability of a variety of clinically employed drugs (Barone *et al*., 2001; Breidenbach *et al*., 2000b; Fugh-Berman, 2000; Johne *et al*., 1999; Miller, 2000; Piscitelli *et al*., 2000; Ruschitzka *et al*., 2000; Vogel, 2001). In this report, both SJW and HYP caused a dramatic increase in P-gp expression after chronic exposure of intestinal cells in an *in vitro* system. Rhodamine 123 cell accumulation assays demonstrated that extended SJW exposure increased P-gp activity (Table 1). Acute inhibition studies in the same cell model demonstrated that rhodamine 123 efflux appears to be P-gp dependent (being affected by known P-gp inhibitors, but not MRP1 inhibitors) (Figure 3). Previous *in vitro* studies, exploring the potential mechanism of the SJW drug interaction, have focused on CYP3A induction *via* steroid receptors (Moore *et al*., 2000; Wentworth *et al*., 2000). However, liver microsome studies have demonstrated that both SJW and HYP are fairly potent CYP3A inhibitors (Obach, 2000), while neither reached an IC_{50} with respect to P-gp mediated transport activity in Caco-2 monolayers (Figure 5). In LS-180V cell accumulation assays, HYP had no inhibitory effect on rhodamine 123 efflux and SJW only caused modest inhibition (Figure 3). Mild inhibition of P-gp activity after acute exposure to SJW, followed by significant induction of P-gp mediated activity with chronic exposure, was observed in this *in vitro* study. This effect was mirrored in a digoxin/SJW clinical study. Digoxin C_{max} demonstrated a trend of transient increase (~20%) after 1 day of SJW oral dosing, but decreased more substantially and continually with chronic administration (33% decrease after 10 days SJW administration) (Johne *et al*., 1999).

SJW extracts may contain hundreds of biologically active compounds. While the exact content of commercially available preparations may vary, calibration to HYP content is a widely accepted index. The 0.3% HYP content of the SJW extract used in all experiments was verified by HPLC analysis (0.25–0.33%). While multiple compounds within SJW may contribute to P-gp induction, it appears that HYP, within the extract, may be mostly responsible for the P-gp induction (100 μg ml^{-1} SJW contains 0.6 μM HYP). HYP appears to be a significant contributor, accounting for a major portion of the induction in our *in vitro* analysis (Figure 2). Considering the 40 h half-life of HYP (Kerb *et al*., 1996), molecular stability and maintenance of steady-state concentrations (even with moderate compliance) may contribute to the development of P-gp

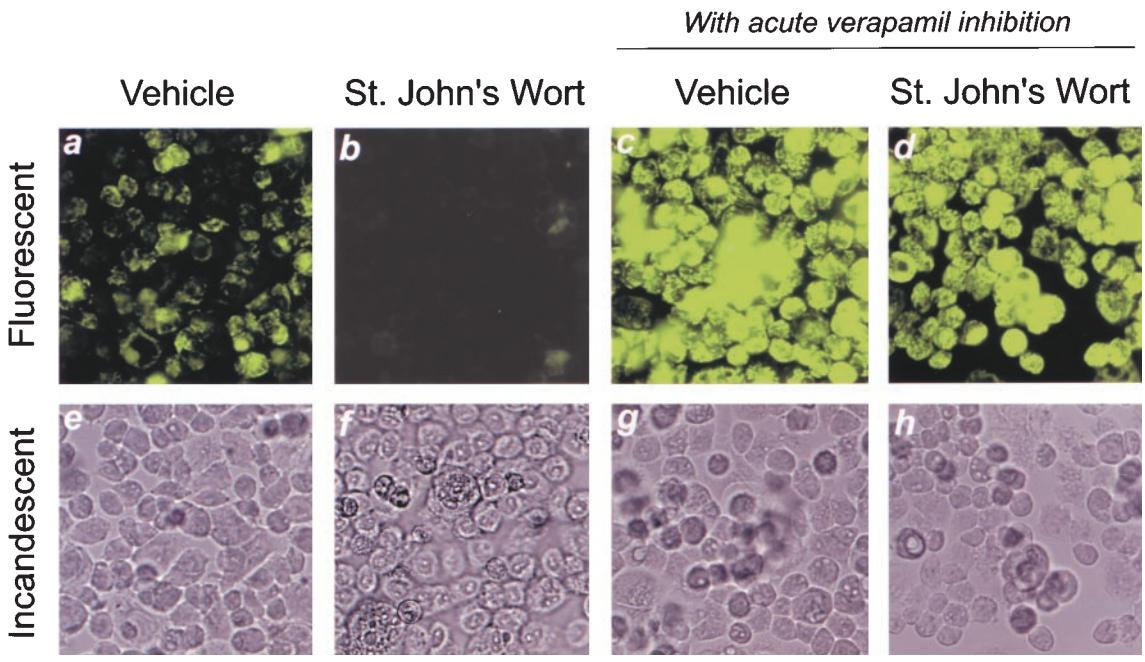

Figure 4 Cell accumulation fluorescence microscopy. Rhodamine 123 cellular accumulation in SJW induced and vehicle control cells was visualized by epi-fluorescence microscopy (a,b,c,d). Incandescent light images of the same field, in each condition, were taken to demonstrate similar cell density (e,f,g,h). Cells were treated with 0.5% DMSO vehicle (a,e) or 100 μg ml^{-1} SJW (b,f). Each image was acquired under identical conditions (200×). Control experiments under the same conditions were performed using 100 μM verapamil acutely, a validated P-gp inhibitor (c,d). Representative fields are shown. Scale bar = 100 μ.

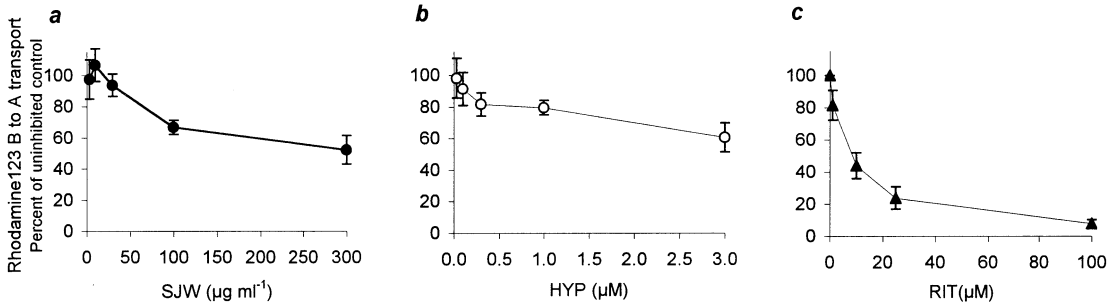

Figure 5 Acute inhibition of P-gp mediated transport in Caco-2 cell monolayers. Inhibition of P-gp mediated rhodamine 123 transport across Caco-2 monolayers by (a) SJW (3, 10, 30, 100, 300 μg ml^{-1}), (b) HYP (0.03, 0.1, 0.3, 1, 3 μM) and (c) ritonavir (1, 10, 25, 100 μM) was assessed by comparison to vehicle control. Data represents rhodamine 123 transport from the basolateral to the apical chamber ($n = 3$) as a per cent of the uninhibited control. Rhodamine transport from the apical to basolateral chamber was less than 5% of basolateral to apical transport in all experiments.

induction with chronic SJW use. Steady-state concentrations of HYP, with 300 mg SJW three times daily, reach greater than 0.03 μM (15 μg l^{-1}) plasma levels. Intraluminal concentrations are most likely much higher (Johne *et al*., 1999; Kerb *et al*., 1996). Considering the substantial induction of P-gp protein (Figure 2c) and the minimal inhibition of P-gp mediated activity (Figure 5b) by HYP shown in this report, HYP and pseudo-HYP compounds within SJW may contribute to clinically significant P-gp induction *in vivo*.

Rodent and human studies have suggested both P-gp and CYP3A induction to explain the SJW drug interaction (Durr *et al*., 2000; Piscitelli *et al*., 2000; Roby *et al*., 2000; Ruschitzka *et al*., 2000). Clinical studies have demonstrated that SJW does increase metabolite formation of CYP3A specific 6-β-hydroxycortisol (Roby *et al*., 2000) as well as demethylation of erythromycin (Durr *et al*., 2000), suggesting hepatic CYP3A induction. However, while SJW has been reported to induce hepatic CYP3A liver expression (Durr *et al*., 2000; Roby *et al*., 2000), no clinical reports of increased clearance of IV medication appear in the literature. In addition, when dexamethasone induces both CYP3A and P-gp in rats, *in vitro* liver and intestinal microsomal metabolism of indinavir is increased 10 fold and 3 fold, respectively (Lin *et al*., 1999). Accordingly, oral bioavailability of indinavir was reduced 50%, while *in vivo* clearance of IV administered drug was largely unaffected (Lin *et al*., 1999). These findings suggest that local induction of P-gp or CYP3A in the intestine is an explanation for clinically significant decrements in oral bioavailability.

While most drugs reported to be affected by the use of SJW are substrates for both CYP3A and P-gp, digoxin is not metabolized by CYP3A (Lacarelle *et al*., 1991) and its bioavailability is reduced by concurrent use of SJW (Durr *et*

al., 2000; Johne et al., 1999). Previous studies have demonstrated that rifampin mediated induction of P-gp can cause significant reduction of digoxin and talinolol oral absorption, demonstrating a clinically important drug interaction not due to alterations in cytochrome P450 mediated metabolism (Greiner et al., 1999; Westphal et al., 2000). Direct comparison of CYP3A and P-gp induction by SJW is difficult due to variation in models of study. In rodent studies in which rats were given 1000 mg kg^{-1} SJW daily by oral gavage for 14 days, induction of intestinal P-gp and CYP3A2 was 3.8 fold and 2.5 fold, respectively. In corresponding clinical studies in which humans were given 900 mg SJW orally for 8 days, induction of intestinal P-gp and CYP3A4 was 1.4 fold and 1.5 fold, respectively (Durr et al., 2000). In both cases, dose response was not explored. However, intestinal concentrations are likely to reach the mg ml^{-1} range, and might saturate the induction response of both proteins (Durr et al., 2000). Based on the data presented in this report, only 25 $\mu g\ ml^{-1}$ of SJW or 0.3 μM HYP were required to double the amount of P-gp immunoreactive protein, while 300 $\mu g\ ml^{-1}$ saturated the response at approximately 4 fold induction. These values appear very similar to those found in rodent studies (Durr et al., 2000). Although CYP3A induction is a partial explanation of SJW's effects on co-administered medications (Durr et al., 2000; Moore et al., 2000; Roby et al., 2000; Wentworth et al., 2000), P-gp induction may also contribute independently.

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