ABSTRACT:
Continuous use of St. John’s wort decreases the bioavailabilities of a variety of drugs. This interaction is attributed to the induction of cytochrome P450 3A4 and/or P-glycoprotein. In this study, we aimed to examine the chronic effects of St. John’s wort and its constituents, hyperforin and hypericin, on the expression and function of P-glycoprotein in an intestinal cell line, LS 180. We also examined the acute inhibitory effect of St. John’s wort on P-glycoprotein by using LLC-GAS-COL150 cells, which overexpress P-glycoprotein. St. John’s wort and hypericin but not hyperforin increased the expression of P-glycoprotein in LS 180 cells. Removal of St. John’s wort resulted in a restoration of P-glycoprotein level within 48 h. The content of hyperforin in St. John’s wort extract was high enough to induce P-glycoprotein, suggesting that the induction of P-glycoprotein by St. John’s wort can be almost attributable to hyperforin. The LS 180 cells chronically exposed to St. John’s wort or hyperforin exhibited the increase in the function of P-glycoprotein assessed by the efflux of digoxin, and the activities correlated well with P-glycoprotein level. On the other hand, St. John’s wort and its two constituents did not show any acute effect on P-glycoprotein-mediated transport of digoxin. St. John’s wort induced P-glycoprotein in vitro that functions as a drug efflux pump. Hyperforin is considered to be a primary cause of the inductive effect of St. John’s wort. Long-term administration of St. John’s wort may cause clinically significant decrease in the plasma concentrations of P-glycoprotein substrates.

St. John’s wort, a traditional herb, has been used as an anti-inflammatory and antidepressive. Recently, St. John’s wort extract has been mainly investigated for its therapeutic effects on mood disorders, and its antidepressive action has been demonstrated (Linde et al., 1996; Wheatley, 1998). Nowadays, St. John’s wort extract is one of the most widely prescribed antidepressants in Germany and also became common in the United States as an over-the-counter drug.

With an increase in the use of St. John’s wort, recent efforts have been made to understand the pharmacokinetic characteristics, including drug interaction caused by St. John’s wort, itself, and its constituents. It has been reported that chronic treatment with St. John’s wort leads to an increase in the metabolism of several drugs, such as indinavir, an HIV protease inhibitor; cyclosporine, an immunosuppressant; and oral contraceptives (Ernst, 1999; Piscitelli et al., 2000; Ruschitzka et al., 2000). These interactions have been attributed to the induction of cytochrome P450 3A4 in the liver and intestine (Du¨rr et al., 2000; Moore et al., 2000; Roby et al., 2000). However, the area under the concentration curve (AUC) of orally administered digoxin, which is a typical substrate of P-glycoprotein (Su and Huang, 1996) and virtually not metabolized in humans (Lacarelle et al., 1991), was reduced by 25% when St. John’s wort was concomitantly administered (Johe et al., 1999). Subsequently, Du¨rr et al. (2000) demonstrated that repetitive administration St. John’s wort increased the content of P-glycoprotein in human intestine.

P-glycoprotein is ubiquitously expressed in the blood-brain barrier, brush-border membranes of intestinal epithelia, and so on. In the intestine, P-glycoprotein acts as an efflux pump to limit the absorption of xenobiotics and contributes to the extrusion of many drugs. Induction of intestinal P-glycoprotein may lead to a decrease in the bioavailability of P-glycoprotein substrates such as cyclosporine (Greiner et al., 1999; Hamman et al., 2001). It has been reported that treatment with St. John’s wort extract for 14 days resulted in a 1.4-fold increase in the activity of hepatic cytochrome P4503A4, and 1.5- and 1.4-fold increases in the expression of duodenal cytochrome P4503A4 and P-glycoprotein, respectively (Du¨rr et al., 2000). St. John’s wort was shown to induce intestinal P-glycoprotein as potent as rifampicin, a well known inducer of P-glycoprotein, by a factor of 3.5 and significantly decreased the oral bioavailability of digoxin by 35% (Greiner et al., 1999). Because a large number of drugs have been increasingly identified as substrates of P-glycoprotein, St. John’s wort possibly affects the pharmacokinetics of many drugs by inducing P-glycoprotein.

St. John’s wort contains more than two dozen bioactive constituents. The antidepressive activity of St. John’s wort has been attributed to hypericin and pseudohypericin (Butterweck et al., 1998). Recently, another constituent, hyperforin, has been identified to be partially responsible for its clinical efficacy (Chatterjee et al., 1998). Hypericin and hyperforin have received attention in recent studies with regard to the induction of cytochrome P4503A and P-glycoprotein by St. John’s
wort. Hyperforin, an abundant and lipophilic component of St. John’s wort, has been reported to activate pregnane X receptor, a key transcriptional regulator of cytochrome P4503A4, and, consequently, to increase the expression of cytochrome P4503A4 in human hepatocytes (Moore et al., 2000). Since the activation of pregnane X receptor is also known to increase the transcription of the MDR1 gene, which encodes P-glycoprotein, and results in an increase in the expression of P-glycoprotein (Geick et al., 2001), it is quite conceivable that the potent pregnane X receptor activator, hyperforin, also induces P-glycoprotein. Hyperforin, which does not activate pregnane X receptor (Moore et al., 2000), has been also reported to induce P-glycoprotein at higher concentration in a cell line, LS 180 cells. Therefore, we also aimed to investigate the effects of these two constituents on the functional induction of P-glycoprotein in this study.

In contrast, Wang et al. (2002) and Johne et al. (1999) have demonstrated that a single dose of St. John’s wort increased the maximum plasma concentration (Cmax) of orally administered fexofenadine and digoxin, suggesting that St. John’s wort may inhibit the function of P-glycoprotein in an acute phase. Indeed, Perloff et al. (2001) showed the possibility that St. John’s wort and hypericin inhibit P-glycoprotein, to an even weaker extent, in a human colon adenocarcinoma cell line, Caco-2.

The objective of this study is to investigate the potency of St. John’s wort to induce and/or inhibit the function of P-glycoprotein, and the relationship between the increases in the protein level and the efflux function of P-glycoprotein in a quantitative manner.

Materials and Methods

Chemicals and Antibodies. St. John’s wort extract (including 0.3% of total naphthodianthrones, in which pseudohypericin is the main constituent usually present in 2- to 4-fold higher amounts than hypericin (Brantner et al., 1994)) was a gift from Matsunaruyakko Ltd. (Aichi, Japan). Rifaxipin was purchased from Merck Ltd. (Tokyo, Japan). Hypericin was purchased from Alexis Inc. (San Diego, CA), dissolved in dimethyl sulfoxide to make the concentration of 20 mM, and stored at −20°C. Clarithromycin was a gift from Taisho Pharmaceutical Co. Ltd. (Tokyo, Japan). [3H]Digoxin (1.0 nCi/ml) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Mouse monoclonal antibody C219 was purchased from TFB Inc. (Tokyo, Japan), [14C]actin was obtained from Sigma-Aldrich (St. Louis, MO), and peroxidase-conjugated sheep affinity-purified antibody to mouse IgG was obtained from Valeant Pharmaceuticals Inc. (Costa Mesa, CA). The enhanced luminol reagent and oxidizing reagent were obtained from PerkinElmer Life and Analytical Sciences. All other reagents used were of the highest purity and commercial availability.

Cell Line and Growth Conditions. The human colon adenocarcinoma cell line, LS 180 was selected as a model of intestinal epithelium. Another human colon adenocarcinoma cell line, Caco-2, is also available and used to investigate the characteristics of intestinal absorption of drugs. However, Caco-2 does not functionally express pregnane X receptor (Thummel et al., 2001); thus, we considered Caco-2 not suitable to investigate the St. John’s wort-mediated induction of P-glycoprotein.

LS 180 was purchased from American Type Culture Collection (Manassas, VA) and grown in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin, at 37°C in 5% CO2. The porcine kidney epithelial cell line, LLC-PK1, and the MDR1-transfected cell line, LLC-GA5-COL150 (Tani-gawara et al., 1992), were purchased from Riken Gene Bank (Ibaraki, Japan) and grown in Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, at 37°C in 5% CO2. Colchicine (150 ng/ml) was added to the medium for the LLC-GA5-COL150 cell line.

St. John’s Wort Preparation. St. John’s wort extract (20, 50, or 75 mg) was transferred to a glass vial, and 1 ml of ethanol was added. After agitation for 30 min, the mixture was transferred to a polyethylene tube and centrifuged at 400g for 5 min. The supernatant was centrifuged again at 400g for 30 min. The supernatant was stored at −20°C and was added to the culture medium or buffer as 1000× stock to make the indicated concentrations (20, 50, or 75 μg/ml).

Isolation of Hyperforin. Ten grams of St. John’s wort extract was suspended into n-hexane (500 ml) for 1 h by stirring at ambient temperature, and then the mixture was placed at 4°C overnight. The mixture was filtered with filter paper, and the filtrate was dried by a rotary evaporator under reduced pressure. The preparation was dissolved in n-hexane (14 ml) and separated on a silica gel column with 7:1 (v/v) n-hexane-acetone as mobile phase. Fractions (10 ml each) were sequentially collected, and the appropriate fractions (as monitored by thin-layer chromatography (TLC)) were mixed and evaporated under reduced pressure to dryness.

The fractions containing hyperforin were identified by TLC on silica gel with 1:1 (v/v) n-hexane-acetic acid (4 ml) and separated on a silica gel column, again, with the same solvent as mobile phase. Fractions (10 ml) were collected; and the appropriate fractions (as monitored by TLC) were mixed and evaporated under reduced pressure, to dryness, and used for the following identification.

Compound identity was confirmed by 1H and 13C NMR spectroscopy as previously described (Erdelmeier, 1998), and data were in accordance with the data in the same literature. The remainder was kept as the authentic sample and dissolved in dimethyl sulfoxide to make the concentration of 8 mM. The sample was stored at −80°C under nitrogen.

Determination of Concentration of Hyperforin in St. John’s Wort Extracts. The concentrations of hyperforin in St. John’s wort extracts were assayed by a high-performance liquid chromatography system, consisting of a pump (LC-6A; Shimadzu, Kyoto, Japan), a UV spectrophotometric detector (SPD-6A; Shimadzu), and a chromatointegrator (C-R6A; Shimadzu).

The separation of hyperforin was carried out by using a reversed phase column (Cosmosil; Nacalai Tesque, Kyoto, Japan) (150 × 4.6 mm i.d., particle size 5 μm). The mobile phase consisted of acetonitrile and water (78:22 v/v) and was pumped at a rate of 1.0 ml/min. Detection was done at a wavelength of 272 nm. Corresponding fractions for each peak were collected and identified by TLC. The retention time of hyperforin was determined to be 28.7 min. A sample of hyperforin obtained from the above isolation experiment was used for calibration.

Cytotoxicity Assay. LS 180 cells were used to investigate the effects of drug treatment on the expression and function of P-glycoprotein. Cells were seeded in 96-well plates (2 × 103 cells/well) and treated by drugs for 24 or 48 h from day 2. Each group consisted of eight wells. Growth inhibition was evaluated by trypan blue dye, WST-1 [2-(4-lodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium, monosodium salt] (Dojindo Chemical Institute Inc., Kumamoto, Japan) colorimetric assay. Cells were incubated with 10 μM WST-1 solution [0.5 mM WST-1, 20 μM 1-methoxy-5-methylphenazinium methylsulfate (Dojindo Chemical Institute Inc.)] in each well for 30 min. The absorbance was quantitated with a microplate reader (Bio-Rad, Hercules, CA) at wavelengths of 570 nm for reference and 630 nm for test drug. Drug concentrations that did not significantly inhibit the formation of dye were used in the P-glycoprotein induction studies.

LCL-PK1 cells and LLC-GA5-COL150 cells were used to investigate the effect of drugs on the transcellular transport of [3H]digoxin mediated by P-glycoprotein. Cells were seeded in 96-well plates (1.35 × 104 cells/well) and grown for 4 days before treatment with drugs for 1 h. Growth inhibition was evaluated after cells were incubated with WST-1 solution for 3 h. Drug concentrations that did not significantly inhibit the formation of dye were used in the transport studies.

Induction of P-glycoprotein. LS 180 cells were seeded in 57-mm dishes (1 × 105 cells/dish) for Western blot assay and four-well plates (1 × 105 cells/well) for efflux studies. From day 2 or day 3, cells had been exposed to the test drugs for 48 or 24 h, respectively. After the drug treatment, the cells were harvested for Western blotting or submitted to the efflux studies under drug-free buffer. To investigate the time course of de-induction, cells were additionally cultured for 24 or 48 h after the removal of test drugs.

Western Blotting. LS 180 cells were harvested, lysed with 0.1% Nonidet P-40 (in 50 mM Tris-HCl buffer, 350 mM NaCl, 5 mM NaF, 10 μg/ml buffer as 1000× stock to make the indicated concentrations (20, 50, or 75 μg/ml).
leupeptin, 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaN₃, and centrifuged, and the supernatant was collected. The total protein concentration was determined according to Lowry et al. (1951) using bovine serum albumin as a standard. An aliquot of protein (150 μg or 10 μg) was loaded in each lane to detect the expression of P-glycoprotein or β-actin, electrophoresed on 7.5% SDS-polyacrylamide gel by the method of Laemmli (1970), and transferred to a 0.2-μm pore size Immobilon Transfer Membrane (Millipore Corporation, Bedford, MA). For immunoblotting, the membranes were blocked with 5% nonfat powdered milk in 20 μM Tris-HCl, 135 mM NaCl, 0.1% Tween 20 at 4°C for 2 h. The blots were incubated with MAb C219 or anti-β-actin monoclonal antibody for 2 h, then with peroxidase-conjugated sheep affinity-purified antibody to mouse IgG as a secondary antibody for 40 min, and washed five times with 1% nonfat powdered milk in 20 μM Tris-HCl, 135 mM NaCl, 0.1% Tween 20 buffer. All washing and incubation steps were performed at ambient temperature. P-glycoprotein and β-actin were detected with the enhanced chemiluminescence system according to the manufacturer’s instructions (PerkinElmer Life and Analytical Sciences). Blots were then exposed to a computer Image Reader (LAS-1000 Lite for LAS-1000 plus version 1.1; Fuji Photo Film Co. Ltd. Tokyo, Japan), and the proteins were quantified with Image Gauge Version 3.41 (Fuji Photo Film Co. Ltd.) for Macintosh. Preparations from control LS 180 cells within a wide total protein range were used to generate relative calibration curves for P-glycoprotein and β-actin to confirm that protein contents of the preparations from drug-treated cells were comparable to their respective controls (data not shown).

The Efflux of [3H]Digoxin. After the removal of the drugs, the LS 180 cells were incubated with buffer for 4 h at 37°C in an atmosphere containing 5% CO₂. Cells were then preincubated for 4 h with 50 nM [3H]digoxin. After incubation, the efflux experiment was initiated by replacing the [3H]digoxin containing buffer with drug-free buffer. To stop the efflux of [3H]digoxin, cells were washed with ice-cold buffer (4°C) at 0, 0.5, 2, or 5 min after the start of efflux, dissolved with NaOH, and neutralized with HCl. The samples were mixed with scintillation fluid (Clearsol I; Nacalai Tesque), and the radioactivity appearing in the opposite side was measured after 15, 30, 45, and 60 min. The samples were mixed with scintillation fluid (Clearsol I; Nacalai Tesque), and the radioactivity was measured by a liquid scintillation counter (LS6500; Beckman Coulter, Fullerton, CA) to determine the intracellular amount of [3H]digoxin. The amount of protein in each well was measured by the method of Lowry et al. (1951).

A standard first-order efflux model could appropriately describe the time courses of the intracellular amount of [3H]digoxin. The equation for this model is given as follows: 

\[ X(t) = A \cdot e^{-kt} + (100 - A) \]

where \( X(t) \) is the intracellular amount of [3H]digoxin at time \( t \) (percentage of the amount at 0 min); \( t \) is the time after the start of the efflux (minutes); \( A \) is the amount of [3H]digoxin that is subject to efflux when the efflux reaches the steady state (percentage of the amount at 0 min); and \( k \) is an efflux rate constant (min⁻¹).

Transcellular Transport of [3H]Digoxin. LLC-PK1 cells and LLC-GAS-COL150 cells were seeded in polycarbonate membrane filters (10 μm pore size) and grown for 1 week. The day before the experiment, the medium was replaced with colchicine-free medium. Cells were incubated with buffer for 4 h at 37°C in an atmosphere containing 5% CO₂. Then, [3H]digoxin was added to the apical or basal side of the dish with or without test drug, which was added to both sides. The radioactivity appearing in the opposite side was measured after 15, 30, 45, and 60 min. Filters were taken out, dissolved with NaOH, and neutralized with HCl. The samples were mixed with scintillation fluid (Clearsol I; Nacalai Tesque), and the radioactivity was measured by a liquid scintillation counter (LS6500; Beckman Coulter) to determine the amounts of [3H]digoxin in each well. The amount of protein in each well was measured by the method of Lowry et al. (1951).

Statistics. Data are expressed as the mean ± S.E.M. Differences between groups and their respective vehicle controls were evaluated by Student’s t test or analysis of variance, followed by Bonferroni’s t test.

Results

Induction of P-glycoprotein by St. John’s Wort Extracts. St. John’s wort extracts induced P-glycoprotein in a time- and dose-dependent manner on the LS 180 cells (Fig. 1). Rifampicin, a well known P-glycoprotein inducer, which was used as a positive control, also increased the expression of P-glycoprotein in a time-dependent manner.

De-induction of P-glycoprotein after the Treatment with St. John’s Wort Extract. The level of P-glycoprotein decreased after the removal of St. John’s wort extract (75 μg/ml) or 10 μM rifampicin in a time-dependent manner and returned to the untreated level at 48 h after removal (Fig. 2).

Effects of Hyperforin and Hypericin on the Expression of P-glycoprotein. Hyperforin caused a time- and dose-dependent induction of P-glycoprotein in the LS 180 cells as well as rifampicin, whereas hypericin did not induce P-glycoprotein under the current experimental conditions (Fig. 3).

Induction of P-glycoprotein by Hyperforin in St. John’s Wort Extract. To determine the extent of the contribution of hyperforin to the induction of P-glycoprotein by St. John’s wort extract, we analyzed the concentration of hyperforin in the St. John’s wort extracts used in the present study. The concentration of hyperforin in 75 μg/ml St. John’s wort extract was 1.06 μM. Since 1 μM hyperforin was equipotent to 75 μg/ml St. John’s wort extract in the induction of P-glycoprotein (Fig. 4), the induction of P-glycoprotein by St. John’s wort extract is almost thoroughly attributable to hyperforin.

The Efflux of [3H]Digoxin from the Cells Treated with St. John’s Wort Extract, Hyperforin, and Hypericin. The efflux rate of [3H]digoxin was increased by St. John’s wort extract treatment in a concentration-dependent manner in the LS 180 cells (Fig. 5). Rifampicin also increased the efflux rate of [3H]digoxin and resulted in a decreased accumulation of [3H]digoxin in the cells. Increased efflux of [3H]digoxin was also observed in the hyperforin-treated cells, but not in the hypericin-treated cells (Fig. 6). There were significant correlations between the efflux rate constants of [3H]digoxin and P-glycoprotein levels in LS 180 cells treated with test drugs or vehicle (Fig. 7).

Effects of St. John’s Wort Extract, Hyperforin, and Hypericin on P-glycoprotein-Mediated Transcellular Transport of [3H]Digoxin. Table 1 represents the cytotoxicities of St. John’s wort extract, hyperforin, and hypericin in LLC-PK1 and LLC-GAS-COL150 cells assessed by WST-1 assay. The highest concentrations of St. John’s wort extract, hyperforin, and hypericin that did not significantly
immunoblots were quantified by densitometry and the results of P-glycoprotein/indicated times. Immunoreactive protein (P-glycoprotein and 

\[ \text{P-gp} \] 

or St. John’s wort extract-treated LS 180 cells. After exposure to 10 \( \mu \text{M} \) rifampicin for 48 h or 75 \( \mu \text{g/ml} \) St. John’s wort extracts (SJW) for 24 h, cells were taken out of drugs for the times indicated prior to harvesting. Immunoreactive protein [P-glycoprotein (P-gp) and \( \beta \)-actin] was detected by Western blotting (top). The immunoblots were quantified by densitometry, and the results of P-glycoprotein/\( \beta \)-actin for each sample are expressed as a percentage of the respective control cells for each time point (bottom). Closed circles, P-glycoprotein expression in rifampicin-treated cells; closed triangles, P-glycoprotein expression in St. John’s wort extract-treated cells.

The basolateral-to-apical transport of \([^{3}\text{H}]\)digoxin was markedly higher than the opposite transport across the \( \text{MDR1} \)-transfected cell line, LLC-GA5-COL150 (Figs. 7 and 8), whereas there were no directional differences in the transcellular transport of \([^{3}\text{H}]\)digoxin across LLC-PK1 cells. In the presence of 500 \( \mu \text{M} \) clarithromycin, a well known inhibitor for P-glycoprotein, the transcellular transport of \([^{3}\text{H}]\)digoxin from the basolateral to the apical side of LLC-GA5-COL150 cells was significantly inhibited, whereas the transport in the opposite direction was increased (Figs. 7 and 8). St. John’s wort extracts and its two ingredients, hyperforin and hypericin, did not affect P-glycoprotein-mediated transcellular transport of \([^{3}\text{H}]\)digoxin across the LLC-GA5-COL150 monolayers (Figs. 7 and 8).

**Discussion**

In recent years, it has become increasingly reported that the use of St. John’s wort extract may decrease plasma concentrations and/or therapeutic effects of drugs such as digoxin, indinavir, cyclosporine, oral contraceptives, theophylline, and warfarin (Ernst, 1999; Johne et al., 1999; Piscitelli et al., 2000; Ruschitzka et al., 2000). This herb-drug interaction is considered to be caused by the induction of cytochrome P450 3A4, cytochrome P450 1A2, and/or P-glycoprotein drug interaction is considered to be caused by the induction of cytochrome P450 3A4, cytochrome P450 1A2, and/or P-glycoprotein (Dürr et al., 2000; Moore et al., 2000; Roby et al., 2000). In a clinical study, St. John’s wort reduced the AUC of indinavir by 57% (Piscitelli et al., 2000). Breidenbach et al. (2000) reported that after the start of St. John’s wort, the blood concentrations of cyclosporine dropped by 49% and led to an increase in the risk of acute rejection in 45 patients after kidney or liver transplantation. Since both indinavir and cyclosporine are metabolized by cytochrome P450 3A4, these interactions were attributed to the induction of cytochrome P450 3A4 by St. John’s wort. However, P-glycoprotein also plays an important role in limiting the absorption of these drugs in the intestine (Seifeldin, 1995; Lin et al., 1999). Moreover, St. John’s wort also decreased the AUC of digoxin, a P-glycoprotein substrate but not metabolized by cytochrome P450 enzymes, by 25% (Lacarelle et al., 1991). Taken together, St. John’s wort extract may cause considerable interaction with P-glycoprotein substrates. However, the relationship between the
level of P-glycoprotein and its function has not been investigated in a quantitative manner. This is the first study to clearly demonstrate the in vitro relationship between the protein level and function of P-glycoprotein during the induction by St. John’s wort and its constituents.

In our study, St. John’s wort extract caused a dose- and time-dependent induction of P-glycoprotein in LS 180 cells (Fig. 1). This result was consistent with the previous report (Perloff et al., 2001). Moreover, we first showed in the present study that hyperforin also induces P-glycoprotein by 1 μM, and it is the primary causative constituent of the P-glycoprotein induction (Figs. 3 and 4). Intestinal concentration of St. John’s wort extract is estimated up to 300 μg/ml after oral intake of St. John’s wort extract (Perloff et al., 2001), and standardized dietary supplements of St. John’s wort contain approximately 3% hyperforin, so that the intestinal concentration of hyperforin may exceed 1 μM. Because of the potency for P-glycoprotein induction and the high content of hyperforin, this constituent is considered to play an important role in the P-glycoprotein induction by St. John’s wort extract in vivo. Hypericin did not affect the expression of P-glycoprotein in this study. This result seems inconsistent with the previous report of Perloff et al. (2001), that 0.3 to 3 μM hypericin increased the expression of P-glycoprotein in LS 180V cells. This discrepancy may be explained by the difference in the concentrations of hypericin used. Due to the cytotoxicity, we could not carry out the experiment with higher concentrations of St. John’s wort extract or hypericin. But in actual fact, the intestinal concentration of hypericin may be up to 3 μM (Perloff et al., 2001). Therefore, hypericin may partially contribute to the induction of P-glycoprotein by St. John’s wort extract.

With regard to the reproducibility of the induction as assessed by the P-glycoprotein/β-actin ratio in Western blot, the within-run variation is relatively small as shown in Fig. 4. On the other hand, the between-run variation of the P-glycoprotein induction is estimated to be larger, from the comparison of the effects of 10 μM rifampicin (48-h treatment) among the results of Figs. 1 to 4. Although the concentration- and treatment time-dependence were well preserved in all the experiments, the absolute value of P-glycoprotein/β-actin ratio between runs should be carefully compared. The induction by 10 μM rifampicin for 48 h was more potent than that by 1 μM hyperforin for 24 h in Fig. 3, whereas the opposite order was observed in another run (Fig. 4). The cause of this inconsistency remains unclear. Some subtle factors such as the passage of the cells or growth cycle may possibly affect the extent of induction.

The P-glycoprotein induction by St. John’s wort was reversible (Fig. 2). Removal of an inducer resulted in a decrease in the expression of P-glycoprotein to the untreated level within 2 days. This result was comparable to the case of nifedipine (Herzog et al., 1993). Herzog et al. (1993) have reported that the de-induction of nifedipine-induced mdr1 mRNA in LS 180-derived cells was observed in 8 h and completed in 3 days. The degradation of P-glycoprotein may rapidly occur in the colon carcinoma cells. However, in a clinical study by Bauer et al. (2002), the reversal of the P-glycoprotein induction took about 2 weeks after discontinuation of St. John’s wort comedication. The induction and de-induction may occur more rapidly in vitro than in vivo. St. John’s wort constituent(s) remaining in the cells may
possibly have antagonized the de-induction. However, it is not conceivable that some constituent(s) remained in the cells even at 24 h after removal with a concentration high enough to exert nearly half the initial induction.

St. John’s wort extract induces both cytochrome P450 3A4 and P-glycoprotein in human intestine (Dürr et al., 2000). The induction of cytochrome P450 3A4 by St. John’s wort and hyperforin has been demonstrated to be caused by the activation of pregnane X receptor (Moore et al., 2000), which functions to enhance the transcription of the CYP3A4 gene. Pregnan X receptor also binds to a response element in the promoter of the MDR1 gene and mediates the induction of P-glycoprotein (Geick et al., 2001; Synold et al., 2001). Thus, it is quite likely that St. John’s wort extract increases the transcription of the MDR1 gene via the activation of pregnane X receptor.

The efflux of [3H]digoxin from LS 180 cells was increased by the treatment with St. John’s wort extracts and hyperforin (Figs. 5 and 6). Moreover, significant correlations were observed between the efflux rate constants of [3H]digoxin and the level of P-glycoprotein (Fig. 7). Therefore, the P-glycoprotein induced by St. John’s wort was proved to be functional. This result was consistent with the observation by Perloff et al. (2001) that chronic treatment by St. John’s wort decreased accumulation of rhodamine 123, a P-glycoprotein substrate, in LS 180V cells. Moreover, it is also in accordance with the observation in vivo that St. John’s wort increased P-glycoprotein expression in human intestine and consequently elevated the oral clearance of digoxin (Dürr et al., 2000; Johne et al., 1999). Interestingly, the steady-state level of the intracellular [3H]digoxin before the efflux experiment was not reduced by the P-glycoprotein inducers, although the efflux rate possibly have antagonized the de-induction. However, it is not conceivable that some constituent(s) remained in the cells even at 24 h after removal with a concentration high enough to exert nearly half the initial induction.

St. John’s wort extract induces both cytochrome P450 3A4 and P-glycoprotein in human intestine (Dürr et al., 2000). The induction of cytochrome P450 3A4 by St. John’s wort and hyperforin has been demonstrated to be caused by the activation of pregnane X receptor (Moore et al., 2000), which functions to enhance the transcription of
constant was increased (data not shown). Therefore, we hypothesized that the inducers had simultaneously induced the transporter that is involved in the uptake of digoxin. The human organic anion-transporting polypeptide 8 (OATP8) is considered to transport digoxin (Kullak-Ublick et al., 2001). Therefore, we also investigated the levels of OATP8 mRNA in LS 180 cells after the treatment with P-glycoprotein inducers by reverse transcription-polymerase chain reaction. However, we failed to detect the change in the level of OATP8 mRNA by P-glycoprotein inducers. Other unidentified transport systems may be possibly induced by the inducers simultaneously.

St. John’s wort extract (10 μg/ml and 20 μg/ml) and its two ingredients, hyperforin (1 μM) and hypericin (0.1 μM), did not show any acute inhibitory effect on P-glycoprotein efflux function assessed by the transport of [3H]digoxin (Figs. 8 and 9). This lack of effect may be due to the low concentrations of the agents used in our study. Indeed, higher concentrations of St. John’s wort (30 μg/ml and 300 μg/ml) and hypericin (0.3 μM and 3 μM) have been shown to cause little inhibition on P-glycoprotein function by previous in vitro study (Perloff et al., 2001). Unfortunately, the experiments with higher concentrations were not feasible due to cytotoxicity in our present study. In any case, St. John’s wort may not be a potent P-glycoprotein inhibitor. In clinical studies, coadministration of St. John’s wort caused a significant increase in the Cmax of fexofenadine, and a slight increase in the Cmax of digoxin occurred on the first day of the coadministration with St. John’s wort (900 mg) (Johne et al., 1999; Wang et al., 2002). However, these acute effects were soon abrogated by the chronic treatment, and the 900-mg dose is the upper limit of the recommended dosage range. Therefore, the inhibitory effects of St. John’s wort are unlikely to cause clinically significant drug interactions.

In conclusion, we first demonstrated that hyperforin is the primary causative component of the P-glycoprotein induction by St. John’s wort. Furthermore, the induced P-glycoprotein was demonstrated to function as an effective efflux pump to limit the influx of P-glycoprotein substrate into intestinal cells. We also analyzed the kinetics of the induction and de-induction of P-glycoprotein in vitro and demonstrated that they were almost completed within 48 h. On the other hand, the potencies of St. John’s wort and its constituents for the induction of P-glycoprotein were found to be weaker than those for the induction of P-glycoprotein.

Address correspondence to: Dr. Yasufumi Sawada, Professor, Department of Drug Informatics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: sawada@phar.kyushu-u.ac.jp