INDUCTION AND INHIBITION OF CYTOCHROMES P450 BY THE ST. JOHN’S WORT CONSTITUENT HYPERFORIN IN HUMAN HEPATOCYTE CULTURES

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ABSTRACT:
St. John’s wort extract (SJW) (*Hypericum perforatum* L.) is among the most commonly used herbal medications in the United States. The predominance of clinical reports indicates that SJW increases the activity of cytochrome P450 3A4 (CYP3A4) enzyme and reduces plasma concentrations of certain drugs. Although the inductive effect of SJW on CYP3A4 is clear, other reports indicate that SJW constituents may have, to a small degree, some enzyme inhibitory effects. Therefore, we sought to study the induction and inhibition effects of the constituents of SJW on CYP3A4 in the human hepatocyte model. Moreover, most research has focused on the induction of CYP3A4 by SJW with little attention paid to other prominent drug-metabolizing enzymes such as CYP1A2, CYP2C9, and CYP2D6. To examine the effects of SJW on CYP1A2, CYP2C9, CYP2D6, as well as CYP3A4, hepatocytes were exposed to hyperforin and hypericin, the primary constituents of SJW extract. Hepatocytes treated with hypericin or hyperforin were exposed to probe substrates to determine enzyme activity and protein and RNA harvested. Hyperforin treatment resulted in significant increases in mRNA, protein, and activity of CYP3A4 and CYP2C9, but had no effect on CYP1A2 or CYP2D6. Acute administration of hyperforin at 5 and 10 μM 1 h before and along with probe substrate inhibited CYP3A4 activity. Hypericin had no effect on any of the enzymes tested. These results demonstrate that with chronic exposure, the inductive effect of SJW on drug-metabolizing enzymes predominate, and human hepatocyte cultures are a versatile in vitro tool for screening the effect of herbal products on cytochrome P450 enzymes.

In 2002, sales of botanical supplements in the United States reached nearly $293 million dollars. St. John’s wort accounted for 15 million U.S. dollars in sales, making it the fourth highest grossing botanical supplement (Blumenthal, 2003). Several clinical studies have demonstrated the effectiveness of St. John’s wort compared with conventional therapy in the treatment of mild to moderate depression (Linde et al., 1996; Wheatley, 1997).

Marketed St. John’s wort, an extract of the flowering portion of the plant *Hypericum perforatum* L., is a mixture of a number of biologically active, complex compounds. At 0.3 mg per capsule, the marketed product. The phloroglucinol hyperforin, the most plentiful lipophilic compound in the extract, is a potent reuptake inhibitor of serotonin, norepinephrine, and dopamine (Muller et al., 1998).

Several recent reports have documented decreased blood/plasma levels of cytochrome P450 3A4 (CYP3A4) substrates, such as indinavir and cyclosporin A, in patients concomitantly taking St. John’s wort (Piscitelli et al., 2000; Ahmed et al., 2001). Similar observations have been documented for digoxin, a substrate of the intestinal transporter P-glycoprotein (P-gp4). Additional in vivo evidence has demonstrated that St. John’s wort increased CYP3A4 and P-gp protein levels in rats (Durr et al., 2000).

Both CYP3A4 and P-gp are transcriptionally regulated by the nuclear orphan receptor pregnane X receptor (PXR). After ligand binding in the cytosol, PXR translocates to the nucleus where it heterodimerizes with retinoid X receptor, and then binds to the CYP3A4 promoter, resulting in increased CYP3A4 and P-gp mRNA

4 Abbreviations used are: P-gp, P-glycoprotein; PXR, pregnane X receptor; EROD, ethoxyresorufin; MS, mass spectrometry; HPLC, high-pressure liquid chromatography; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P450, cytochrome P450; PCR, polymerase chain reaction; HH, human hepatocyte donor.
expression. In vitro studies have shown that hyperforin, but not hypericin, is a potent activator of PXR (Moore et al., 2000). In addition to increasing expression of CYP3A4 and P-gp, PXR has also been shown to play a role in CYP2C9 expression (Gerbal-Chaloin et al., 2001).

Although most research has pointed to the ability of St. John’s wort to induce CYP3A4, one report documented the potent inhibition of CYP3A4, CYP2C9, and CYP2D6 by hyperforin and hypericin (Obach, 2000). The objective of our experiments was to use primary cultures of human hepatocytes to characterize the effect of hypericin and hyperforin on CYP1A2, CYP2C9, CYP2D6, and CYP3A4 mRNA expression, protein content, and enzyme activity. We also evaluated the potential of hyperforin to inhibit CYP3A4 enzymes using human hepatocyte cultures.

Materials and Methods

Chemicals. Williams’ E culture medium and medium supplements, dexamethasone and insulin, were obtained from Cambrex BioScience Walkersville, Inc. (Walkersville, MD). Fenclilin G/streptomycin was obtained from Invitrogen (Carlsbad, CA). Rifampicin, phenobarbital, dexamethasone, β-naphthoflavone, hypericin, ethoxyresorufln (EROD), and testosterone were obtained from Sigma-Aldrich (St. Louis, MO). Hyperforin was isolated from St. John’s wort leaf/flower mixtures at the National Center for Toxicological Research. The purified compound was identified by liquid chromatography/mass spectrometry (MS) and nuclear magnetic resonance analysis, and the purity (>98%) was further determined by liquid chromatography/photo diode array method (Liu et al., 2000). 6β-Hydroxytestosterone was obtained from Steraloids (Wilton, NH). Falcon six-well culture plates were obtained from BD Biosciences Discovery Labware (Bedford, MA). Nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate color developing reagent and alkaline phosphatase-conjugated anti-rabbit and anti-goat antibodies were purchased from Bio-Rad (Hercules, CA). Baculovirus-expressed CYP1A2, CYP2C9, CYP2D6, and CYP3A4 were obtained from BD Gentest (Woburn, MA). Antibodies used to detect CYP1A2 (458124), CYP2D6 (458246), and CYP3A4 (458223) were purchased from BD Gentest. CYP2C9 (RDI-CYP2C9ab) antibodies were purchased from Research Diagnostics (Flanders, NJ). Reagents for reverse transcription were purchased from Promega (Madison, WI). Forward and reverse primers for CYP1A2, CYP2C9, CYP3A4, and β-actin were synthesized by Applied Biosystems (Foster City, CA). CYP2D6 forward and reverse primers and TaqMan probe were purchased from Applied Biosystems (assay ID: Hs00164385_m1). All solvents and other chemicals used were of HPLC grade or the highest purity available.

Hepatocyte Isolation. Hepatocytes were isolated from nine human liver donors. Human liver tissue was procured under an institutional review board-approved protocol with support from the liver tissue procurement and distribution system. Table 1 summarizes the demographics of the donors used in induction and inhibition studies. Human hepatocytes were prepared by a three-step collagenase perfusion technique (Strom et al., 1996). If the initial viability was less than 75%, the hepatocytes were subjected to density gradient separation using Percoll to remove nonviable cells (Table 1). Viability of cells was determined by the trypan blue exclusion method and ranged from 70 to 89%. Hepatocytes were plated on Falcon six-well culture plates (1.5 × 10⁴ cells) or P100 (10 × 10⁴ cells) plates, previously coated with rat-tail collagen in Williams’ E medium supplemented with 0.1 μM insulin, 0.1 μM dexamethasone, 0.05% streptomycin, 0.05% penicillin, 0.05% amphotericin B, and 10% bovine calf serum. After cells attached for 4 h, medium was replaced with serum-free medium containing all the supplements described above. Cells were maintained in culture at 37°C in atmosphere containing 5% CO₂ and 95% air. After 24 h in culture, unattached cells were removed by gentle agitation and the medium was changed every 24 h. At 48 and 72 h after plating, cells were exposed to rifampicin (10 μM), dexamethasone (50 μM), β-naphthoflavone (50 μM), hyperforin (0.2 and 1.0 μM), or hypericin (1.0 and 2.5 μM), all dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in medium was 0.1%.

Evaluation of the Cytotoxicity of Hyperforin/Hypericin to Human Hepatocytes. Hepatocytes were exposed to 0, 0.5, 1.0, 2.5, and 5 μM hypericin and 0, 0.2, 1.0, 2.5, and 5 μM hyperforin for 48 h. Following aspiration of media, 10% v/v of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to Williams’ E medium at 96 h of culture and incubated for 30 min. At 30 min, the medium was aspirated and cells washed with Williams’ E culture medium. Isopropanol (same volume as the medium) was then added and shaken gently for 2 min. Two hundred microliters of this solution was transferred to a 96-well plate, and the absorbance was measured at 490 nm.

Measurement of Enzyme Activity. After 96 h in culture, cells were washed with medium that does not contain the supplements described above for 1 h at 37°C. Cells were then exposed to medium containing testosterone (250 μM), flurbiprofen (100 μM), EROD (10 μM), or dextromethorphan (100 μM) for 30 min.

HPLC Measurement of CYP3A4 Activity. The concentration of 6β-hydroxytestosterone in the medium was measured by HPLC as previously described (Kostrobysky et al., 1999) with the following modifications. Fifty microliters of medium was diluted with an equal volume of methanol, spun at 12,000 rpm. One hundred microliters of this solution was injected onto a LiChroSpher 100 RP-18 column (4.6 × 250 mm, 5 μm; Merck, Gibbstown, NJ). 6β-Hydroxytestosterone was eluted in a mobile phase of methanol/water (60:40, v/v) at a flow rate of 1.2 ml/min, and the eluents were monitored at 242 nm.

HPLC Measurement of CYP2C9 Activity. The concentration of 4'-
hydroxylflurbiprofen in the medium was measured by HPLC as previously described (Tracy et al., 1995), with the following modifications. Samples were acidified with 20 μl of H3PO4 and quenched with 200 μl of acetonitrile containing 36 ng of 2-fluoro-4-biphenyl acetic acid (internal standard). After centrifugation at 10,000 rpm for 4 min, 50 μl of sample was injected onto the HPLC system (Waters Alliance 2690XE HPLC system; Waters, Milford, MA). Mobile phase, at a rate of 1 ml/min, consisted of acetonitrile/10 mM KH2PO4, pH 3.0 (40:60) and was pumped through a Brownlee Spheri-5 C18, 4.6 × 100-mm column (PerkinElmer Life and Analytical Sciences, Boston, MA). The metabolite was detected with a fluorescence detector (Waters 474) with an excitation and emission wavelengths of 260 and 320 nm, respectively.

**Luminescent Spectrophotometric Measurement of CYP1A2 Activity.** The activity of CYP1A2 was assessed by measuring the conversion rate of EROD to resorufin as described previously (Pohl and Fouts, 1980). The product resorufin was measured in culture medium after a 30-min incubation, using a PerkinElmer LS 50 B fluorescence plate reader (PerkinElmer Life and Analytical Sciences) at 530-nm excitation and 580-nm emission.

**HPLC-MS Measurement of CYP2D6 Activity.** The concentration of dextrophan in the medium was measured by HPLC-MS. An aliquot of (200 μl) was combined with glycine buffer (1 M, pH 11.3) and extracted with hexane/tert-butyl methyl ether (75:25). The organic layer was transferred to a clean tube and the sample was back-extracted into 0.2 N hydrochloric acid (200 μl). The samples were injected (20 μl) onto the HPLC system, and the eluent was monitored by selected ion monitoring of m/z 258 (dextrophan) and m/z 284 (levallorphan, internal standard) with a triple-quadrupole mass spectrometer operated in electrospray positive ion mode. Chromatography was performed using a Phenomenex Max-RP C12 column (2.0 × 150 mm; Phenomenex, Torrance, CA) and a mobile phase consisting of methanol/water (55:45, v/v) containing 0.1% formic acid, which was delivered isocratically at a flow rate of 0.2 ml/min.

**Evaluation of Acute Effect of Hyperforin on CYP3A4 Activity.** To determine whether hyperforin or one of its metabolites can inhibit CYP3A4 activity, human hepatocytes treated with 1.0 μM hyperforin for 48 h, referred to as chronic (c), were then exposed to the same concentration of hyperforin 1 h before the addition of the probe substrate, referred to as preincubation (p), and along with the substrate, referred to as acute (a) treatment. In addition, untreated hepatocytes were preincubated (p) with hyperforin (1, 5, or 10 μM) for 1 h before addition of substrate and then treated acutely (a) with the same concentration of hyperforin along with the probe substrate. Media and cells were processed in a manner identical to that outlined below.

**Measurement of Immunoreactive Protein.** After removing the medium, cells were harvested in a phosphate buffer (0.1 M, pH 7.4) and stored at −80°C for protein determination (Lowry et al., 1951) and detection of immunoreactive CYP2C9 protein. Immunochromatographic detection of all P450 isomers was performed as previously described (Kostrubsky et al., 1995) using 18 μg of sonicated pooled proteins. CYP3A4 protein was detected with a rabbit antihuman antibody, CYP2C9 protein was detected with a rabbit anti-human CYP2C9 antibody, and CYP1A2 and CYP2D6 were detected with a goat antihuman antibody. A horseradish peroxidase-conjugated anti-rabbit or anti-goat antibody and Nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate-developing reagent were used to visualize the blots.

**Extraction of RNA and Reverse Transcription.** Total RNA was extracted from 10 × 10⁶ cells plated on P100 plates using 3 ml of Trizol reagent (Invitrogen) according to the manufacturer’s instructions. RNA was quantified spectrophotometrically and subjected to agarose gel electrophoresis to assess RNA integrity. After treatment with RNase-free DNase (Promega), 2 μg of RNA was mixed with 0.5 μg of Random Hexamers (Promega), then heated to 70°C for 5 min, then cooled to 4°C. A reaction mixture containing 200 U Moloney murine leukemia virus-reverse transcriptase, 1 mM deoxynucleoside-5′-triphosphates, and 25 U RNasin (Promega) was added to the previous mixture and incubated at 37°C for 60 min. The resulting cDNA was diluted 10-fold and stored at −20°C.

**Real-Time PCR.** Primers for CYP1A2 (Finnnstrom et al., 2001) and CYP3A4 (Bowen et al., 2000) were described previously. Primers for CYP2C9 and β-actin designed using PrimerExpress 1.0 (Applied Biosystems) were 5′-AAT GGA CAT GAA CAA CCC TCA-3′ and 5′-CTC AGG GTT GTG CTT GTC GT-3′, and 5′-AGG CAT CCT CAC CCT GAA GTA-3′ and 5′-CAC AGG CAC CTC ATT GTA GA-3′, respectively. Assays on Demand gene expression product Hs00164385_m1 (Applied Biosystems) were used to detect CYP2D6. PCR was performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using 5 μl of cDNA, 200 pM forward and reverse primers (SYBR green technology; Applied Biosystems), or 1.25 μl of Assays on Demand-mix (TaqMan technology; Roche, Branachburg, NJ) and 12.5 μl of PCR Master Mix (Applied Biosystems) for a total volume of 25 μl. PCR conditions were 50°C for 2 min, 95°C for 10 min, followed by 50 cycles with 15 s at 95°C and 1 min at 60°C. The relative cDNA content was determined from standard curves constructed from serially diluted cDNA and normalized to β-actin in each sample.

**Statistics.** The data were analyzed using a one-way analysis of variance with a post hoc Dunnett’s procedure. The results were considered statistically significant at p ≤ 0.05.

**Results**

**Assessment of Hyperforin- and Hypericin-Mediated Cytotoxicity.** Hepatocytes prepared from two donors were exposed to hypericin at 0.5, 1.0, 2.5, and 5.0 μM and hyperforin at 0.2, 1.0, 2.5, and 5.0 μM for 48 h to assess the effect on the hepatocyte mitochondrial activity by an MTT assay. Concentrations of hypericin at 5.0 μM and hyperforin at 2.5 μM exhibited decreased mitochondrial activity as assessed by MTT reduction as shown in Fig. 1.

**Effect of Hypericin and Hyperforin on Enzyme Activity.** Because of the toxicity seen at concentrations of 5.0 μM and 2.5 μM for hypericin and hyperforin, respectively, we examined the effect of hypericin and hyperforin on CYP3A4 activity as measured by 6β-hydroxytestosterone formation rate at 1.0 and 2.5 μM, and 0.2 and 1.0 μM, respectively. Results for treatment of three cultures are shown in Table 2, and one culture (HH921) is displayed in Fig. 2. In HH906 (Table 2), the rate of formation of 6β-hydroxytestosterone in the DMSO-treated cells was 0.10 ± 0.01 nmol/min/mg protein. Treatment with hyperforin at 0.2 and 1.0 μM resulted in a 3.3- and 7.9-fold increase in the formation rate of 6β-hydroxytestosterone as compared with DMSO-treated cells, respectively. Rifampicin (10 μM), in the same culture, caused a 9.6-fold increase in 6β-hydroxytestosterone formation rate compared with DMSO-treated cells. Treatment of cells with hyperforin at 0.2 and 1.0 μM resulted in a 2- and 3.2-fold increase in 6β-hydroxytestosterone formation rate compared with DMSO-treated cells. In all cultures, no
Effect of hypericin (Hpc) and hyperforin (Hpf) on CYP3A4 activity.

Table 2

<table>
<thead>
<tr>
<th>Culture ID</th>
<th>CYP3A4 - Testosterone</th>
<th>CYP2C9 - Flurbiprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HH906</td>
<td>HH913</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.10 ± 0.01</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>Rif 10 μM</td>
<td>0.16 ± 0.08</td>
<td>1.67 ± 0.01*</td>
</tr>
<tr>
<td>Hpf 0.2 μM</td>
<td>0.16 ± 0.02</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Hpf 1.0 μM</td>
<td>0.16 ± 0.02</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>Hpc 0.2 μM</td>
<td>0.33 ± 0.03*</td>
<td>0.52 ± 0.11*</td>
</tr>
<tr>
<td>Hpc 1.0 μM</td>
<td>0.79 ± 0.07*</td>
<td>0.84 ± 0.06*</td>
</tr>
</tbody>
</table>

Rif, rifampicin. * significantly different from DMSO-treated cells, p < 0.05.

Fig. 2. Effect of hypericin (Hpc) and hyperforin (Hpf) on CYP3A4 activity.

Human hepatocytes from three donors (HH906, HH913, and HH921) were treated with 0.1, 0.5, and 1.0 μM Hpc and 0.2, 0.5, 1.0, and 1.5 μM Hpf for 48 h. At the end of the time period, cells were incubated with 250 μM testosterone for 30 min. After 30 min, aliquots of medium were analyzed for 6β-hydroxytestosterone by HPLC as described under Materials and Methods. Data shown are from HH921. Each value represents the mean of triplicate treatments with the S.D. indicated by the vertical bars. * significantly different from DMSO-treated cells, p < 0.05.

Significant difference in 6β-hydroxytestosterone formation rate was observed in cells treated with 1.0 or 2.5 μM hypericin.

In HH913 (Table 2), the 4'-hydroxyflurbiprofen formation rate was 0.12 nmol/min/mg protein. Treatment with hyperforin at 0.2 and 1.0 μM resulted in a 1.5- and 1.6-fold increase in the formation rate of 4'-hydroxyflurbiprofen compared with DMSO-treated cells. Treatment of hepatocytes with hyperforin at 0.2 and 1.0 μM resulted in a 1.9- and 1.5-fold increase in 4'-hydroxyflurbiprofen formation rate in HH919 and a 2- and 1.7-fold increase in HH926. Rifampicin (10 μM) in HH913, HH919, and HH926 caused a 2.3-, 2.2-, and 2.9-fold increase in CYP2C9 enzyme activity, respectively. Hypericin treatment at 1.0 and 2.5 μM did not alter CYP2C9 activity in any of the cultures.

CYP2D6 enzyme activity was measured by dextromethorphan to dextrophan formation rate, and CYP1A2 was measured by ethoxyresorufin to resorufin formation rate. In hepatocytes from two donors (HH921, HH926), 0.2 and 1.0 μM hypericin or 1.0 and 2.5 μM hyperforin treatment did not significantly change CYP1A2 or CYP2D6 activity when compared with DMSO only (p < 0.05) (data not shown).

To determine whether hyperforin could inhibit CYP3A4 activity and whether induction or inhibition would predominate following chronic hyperforin exposure, we examined the effect of (c), (p), and/or (a) hyperforin exposure on CYP3A4 activity. Chronic exposure alone of hepatocytes to 1.0 μM hyperforin resulted in a significant increase in 6β-hydroxytestosterone formation rate from 0.21 ± 0.00 to 0.50 ± 0.11 nmol/min/mg as seen in Fig. 3 (p < 0.05). There was no difference in activity when the latter was compared with cells treated with (c)-(p)-(a) hyperforin 1.0 μM. With (p)-(a) hyperforin (1.0 μM) and (a) hyperforin (1.0 μM) there was no change in CYP3A4 activity compared with DMSO. However, 6β-hydroxytestosterone formation rate was significantly decreased with (p)-(a) hyperforin (5.0 μM) and (p)-(a) hyperforin (10.0 μM) from a control value of 0.21 ± 0.01 nmol/min/mg to 0.13 ± 0.03 and 0.11 ± 0.04 nmol/min/mg, respectively (p < 0.05). Ketoconazole was used as a positive control and significantly reduced CYP3A4 activity (p < 0.05).

Effect of Hypericin and Hyperforin on Protein Content. To determine whether the increase in CYP3A4/S and CYP2C9 enzymatic activity resulted from increased immunoreactive protein, Western blot analysis was performed. In Fig. 4, Western blot analysis showed an increase in immunoreactive CYP3A4/S in hepatocytes treated with 0.2 and 1.0 μM hypericin when compared with DMSO-treated controls. Similarly, hyperforin (0.2 and 1.0 μM) resulted in an increase in CYP2C9 immunoreactive protein. Rifampicin (10 μM) treatment resulted in increases in both CYP3A4 and CYP2C9 protein content. CYP2D6 and CYP1A2 protein levels were not increased by hyperforin (0.2 and 1.0 μM). Hypericin (1.0 and 2.5 μM) did not increase the immunoreactive protein of any of the enzymes.

Effect of Hypericin and Hyperforin on mRNA Expression. To determine whether the increase in CYP3A4/S and CYP2C9 activity resulted from increased mRNA expression, real-time PCR analysis was performed. Hepatocytes treated with hypericin 0.2 and 1.0 μM resulted in a significant increase in mRNA expression of CYP3A4 and CYP2C9 (Fig. 5). A small increase (2-fold) in CYP1A2 expression occurred with hypericin treatment. Cells treated with hypericin showed a small decrease in the expression of CYP1A2 and CYP2C9 expression.

Discussion

Primary cultures of human hepatocytes offer a number of advantages over other systems commonly used in drug metabolism research. Hepatocytes are intact systems containing all the necessary cofactors for the oxidative, reductive, and conjugative metabolism of xenobiotics. Yet their simplicity compared with liver slices, whole-perfused organs, or entire animal systems allows for the elimination of confounding factors such as blood flow and blood protein binding.

In this study we have shown that human hepatocytes exposed chronically to the St. John’s wort constituent hyperforin (up to 1.0 μM) had increased CYP3A4 mRNA expression, protein content, and enzyme activity. The capacity of hyperforin to induce CYP3A4, EC_{50} = 0.5 μM, was comparable with that of rifampicin, EC_{50} = 0.5 μM (Sahi et al., 2000), albeit to a lower extent (lower E_{max}). However,
**Fig. 3.** Effect of 48 h chronic (c), 1 h pretreatment (p), and acute (a) hyperforin (Hpf) exposure on testosterone CYP3A4.

Hepatocytes from two human donors (HH943, HH944) were treated with 0, 1, 5, and 10 μM Hpf. (c), 48 h treatment with Hpf; (p), Hpf added for 1 h before the addition of testosterone; (a), Hpf added along with testosterone; (p-a), Hpf added for 1 h before the addition of testosterone followed by Hpf added along with testosterone. Data shown are from HH943. *, significantly different from DMSO-treated cells, p < 0.05. Ktz, ketoconazole; c, chronic; p, pretreatment for 1 h; a, acute.

**Fig. 4.** Effect of hypericin (Hpc) and hyperforin (Hpf) on the P450 protein content.

Hepatocytes from two human donors (HH906, HH913) were treated with 0, 1.0, and 2.5 μM Hpc and 0, 0.2, and 1.0 μM Hpf for 48 h. Immunodetectable CYP3A4 (A), CYP2C9 (B), CYP1A2 (C), and CYP2D6 (D) isoform levels were analyzed in pooled sonicates of whole cells harvested in phosphate buffer as described under Materials and Methods. Sonicated protein (18 μg) was applied per well.

**Fig. 5.** Effect of hypericin (Hpc) and hyperforin (Hpf) on P450 mRNA levels.

Hepatocytes from three donors (HH926, HH943, HH1002) were treated with 0, 1.0, and 2.5 μM Hpc and 0, 0.2, and 1.0 μM Hpf for 48 h. Real-time PCR was performed on CYP3A4 (A), CYP2C9 (B), CYP1A2 (C), and CYP2D6 (D) generated cDNA and data pooled for statistical analysis. *, significantly different from DMSO-treated cells, p < 0.05.
cells treated chronically with another prominent St. John’s wart constituent, hypericin, did not show any change in the mRNA expression or activity of this enzyme. These data are consistent with reports that St. John’s wort administration along with other CYP3A4 substrates, such as cyclosporin and indinavir, results in decreased plasma or blood levels of these drugs (Piscitelli et al., 2000; Ahmed et al., 2001) but are inconsistent with other studies that showed CYP3A4 inhibition by St. John’s wort constituents in expressed human enzymes (Obach, 2000) or no effect on these enzymes (Noldner and Chatterjee, 2001).

When administered to humans as a single 900-mg dose, St. John’s wort increased the bioavailability of fexofenadine, a P-glycoprotein substrate, indicating inhibition of the intestinal transporter (Wang et al., 2002). However, when administered at 300 mg three times a day for 14 days, St. John’s wort caused a significant increase in fexofenadine clearance compared with single-dose therapy, consistent with induction of P-glycoprotein. The latter is in line with the ability of hyperforin to activate the nuclear factor PXR with subsequent transcriptional activation of P-glycoprotein expression (Moore et al., 2000). The similarity of transcriptional regulation of P-gp and CYP3A4, along with an overlapping substrate/inhibitor profile, lends further credence to the hypothesis that CYP3A4 and P-gp are capable of being inhibited by a constituent of St. John’s wort before onset of the inductive effect on CYP3A4 and P-gp that occurs with chronic exposure.

Chronic (48 h) exposure of human hepatocytes to hyperforin is limited to concentrations below 1.5 μM due to cytotoxicity. However, it is possible to expose human hepatocytes to higher concentrations of hyperforin (5 and 10 μM) for short durations, such as 1 h before the addition of the probe compound. Because exposure is limited to 1 h, any increase in P450 protein expression is negligible, and the direct effect of hyperforin on the enzyme can be documented. Indeed, the presence of hyperforin levels 5 and 10 times greater than that capable of inducing the enzyme resulted in an inhibition of CYP3A4 activity (Fig. 5). The study conducted by Obach (2000) using expressed human P450 enzymes found the Kᵢ value of hyperforin for CYP3A4, CYP2D6, and CYP2C9 to be 0.49, 1.5, and 1.8 μM, respectively. It should be noted that the significant inhibition of CYP3A4 by hyperforin occurred at 3 μM, a concentration that is greater than that required for induction in primary cultures of human hepatocytes. Furthermore, it has been shown in a PXR binding assay that hyperforin is actually a more potent PXR ligand than rifampicin, yet rifampicin results in greater expression of CYP3A4 mRNA human hepatocytes (Moore et al., 2000). The latter indicates that in an intact cellular system, seen either in cell culture or in vivo, hyperforin is in some way handled by the cell, perhaps as a substrate for a membrane transporter, metabolized to an inactive metabolite or partitions into hepatocytes to a lesser degree. Because the former inhibition studies were conducted in a microsomal system, passage across the outer cellular membrane does not occur. These factors may explain why our studies required hyperforin concentrations 5 to 10 times the inducing concentrations to document enzyme inhibition.

Interestingly, the potent PXR ligand rifampicin has also been shown to induce CYP2C9 mRNA in primary cultures of human hepatocytes, albeit to a lower extent than its induction of CYP3A4 (Gerbal-Chaloin et al., 2001). Logically, hepatocytes exposed to hyperforin, a potent PXR ligand, should demonstrate increased CYP2C9 enzymatic activity and expression. Therefore, our data show that rifampicin (10 μM) treatment resulted in an increase in CYP2C9 mRNA expression, protein content, and enzyme activity. More importantly, for the first time, we have documented an increase in CYP2C9 mRNA expression, protein content, and activity in human hepatocytes exposed to hyperforin at concentrations identical to those that cause an increase in CYP3A4 activity.

Although documentation of St. John’s wort’s role in CYP2C9-mediated drug metabolism is limited, one case report has documented a reduced anticoagulant effect of warfarin, a substrate of CYP2C9, in patients taking St. John’s wort (Yue et al., 2000), and another study showed a reduction in the area under the curve of phenprocoumon, also a substrate of CYP2C9, after St. John’s wort administration (Maurer et al., 1999). However, a study that used tolbutamide as a probe for CYP2C9 failed to show any change in area under the curve compared with placebo in patients administered St. John’s wort extract for 14 days (Wang et al., 2001). The reasons for this apparent incongruence are as yet unknown, but in human hepatocyte cultures, hyperforin clearly results in an inductive effect of CYP2C9.

Hyperforin’s effect on drug-metabolizing enzymes may not be confined to CYP3A4 and CYP2C9 isoforms. Some case reports have indicated a modulation of metabolism of theophylline, metabolized by CYP1A2, and amitriptyline, metabolized by CYP2D6 and CYP1A2, in patients taking St. John’s wort. We observed no significant change in CYP1A2 or CYP2D6 activity (data not shown) or protein content (Fig. 3) in human hepatocytes exposed to hyperforin or hypericin. Based on these data, it is unlikely that the St. John’s wort constituents studied will result in any clinically significant drug interactions in vivo with substrates of CYP1A2 and CYP2D6.

Our studies clearly document the potential for the St. John’s wort constituent hyperforin to induce CYP3A4 and CYP2C9 upon chronic exposure. Combined, both enzymes are responsible for the metabolism of a wide variety of commonly prescribed medications worldwide. Decreased blood plasma concentrations of drugs that may result from induction of these enzymes has the potential to seriously alter desired drug therapy outcomes in patients concurrently taking St. John’s wort. Although we have shown that hyperforin has the potential to inhibit CYP3A4, particularly after single exposure at high concentrations, the inductive effect predominates with chronic exposure. Furthermore, our studies have demonstrated the utility of human hepatocyte cultures in clarifying induction/inhibition discrepancies that are related to how a compound affects a drug-metabolizing enzyme(s).

References

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