Hyperforin-Induced Activation of the Pregnane X Receptor Is Influenced by the Organic Anion-Transporting Polypeptide 2B1

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ABSTRACT

The herbal remedy St. John’s wort (SJW) is used in the treatment of mild depressive symptoms and is known for its drug-drug interaction potential when enhanced expression of CYP3A4 modifies clearance of concomitantly applied substrate drugs. Hyperforin is one constituent of SJW that alters CYP3A4 expression by activation of the nuclear receptor pregnane X receptor (PXR). However, little is known about the transmembrane transport of hyperforin. One membrane protein that modulates cellular entry of drugs is the organic anion-transporting polypeptide (OATP) 2B1. It was the aim of this study to test whether hyperforin interacts with this transport protein. Transport inhibition studies and competitive counterflow experiments suggested that hyperforin is a substrate of OATP2B1. This notion was validated by showing that the presence of OATP2B1 enhanced the hyperforin-induced PXR activation in cell-based luciferase assays. Moreover, in Caco-2 cells transcellular transport of the known OATP2B1 substrate atorvastatin was changed in the presence of hyperforin, resulting in an increased efflux ratio. Eleven commercially available SJW formulations were assessed for their influence on OATP2B1-mediated transport of estrone 3-sulfate and for their impact on CYP3A4 promoter transactivation. The correlation between effect size and the hyperforin content as determined by high-performance liquid chromatography with ultraviolet detection suggested that hyperforin is the major determinant. Our results indicate an interaction between hyperforin and OATP2B1, which is not only known to contribute to hepatocellular uptake but also to intestinal absorption of its substrates. These findings extend the complexity of mechanisms that should be considered when evaluating the interaction potential of SJW preparations.

Introduction

St. John’s wort is an herbal extract of Hypericum perforatum and is commonly taken to treat depressive symptoms. The extract is a complex mixture of structurally diverse constituents, including flavonol glycosides, phloroglucinols, proanthocyanidins, naphthodianthrones, and phenylpropanoids (Nahrstedt and Butterweck, 1997). The naphthodianthrones pseudohypericin and hypericin were originally assumed to be the active components of Hypericum extracts (Suzuki et al., 1984). Accordingly, hypericin became the constituent on which therapeutically used extracts are standardized. However, even though the antidepressive activity is still not fully understood, more recent studies suggest that it is most probably linked to the phloroglucinol hyperforin. (Mennini and Gobbi, 2004).

Beside its antidepressant activity, St. John’s wort is known for its pronounced influence on expression and activity of genes involved in drug metabolism (Soleymani et al., 2017). Indeed, hyperforin enhances expression and activity of CYP3A4, thereby modifying the first-pass metabolism and clearance of concomitantly applied substrates (Wang et al., 2013). Similar results were shown for the efflux transporter P-glycoprotein (P-gp, ABCB1, MDR1), when treatment with St. John’s wort extracts increased intestinal ABCB1 expression, explaining the reduced bioavailability of ABCB1 substrates (Dürr et al., 2000). The underlying mechanism is the activation of the pregnane X receptor (PXR) (Kliwer et al., 2002). PXR is a nuclear receptor functioning as ligand-activated transcription factor of a gene network comprising various proteins involved in drug metabolism, including CYP3A4 (Lehmann et al., 1998), the efflux transporter P-gp (Geick et al., 2001), the multidrug-resistance protein 3 (ABCC3, MRP3) (Aleskunes and Klaassen, 2012), the organic anion-transporting polypeptide (OATP) 1A2 (Meyer zu Schwabedissen et al., 2008), the UDP-glucuronosyltransferase 1a5 (Ugt1a5), and the sulfotransferase 2a2 (Sult2a2) (Aleskunes and Klaassen, 2012). Accordingly, PXR activation in general results in enhanced metabolic activity and increased metabolic clearance. Hyperforin, even if very potent, is not the only activator of PXR, since multiple drugs in clinical use function as activating ligands (Meyer zu Schwabedissen and Kim, 2009). Accordingly, PXR is also called a xenosensor as it senses drug exposure and modulates metabolic activity in response. There are multiple examples

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ABBREVIATIONS: BSP, bromosulfophthalein; CCF, competitive counterflow; E3S, estrone 3-sulfate; HPLC, high-performance liquid chromatography; MDCKII cells, Madin-Darby canine kidney epithelial cells; MRP, multidrug resistance-associated protein; OATP, organic anion-transporting polypeptide; PBS, phosphate-buffered saline; PXR, pregnane X receptor; SJW, St. John’s wort.
in the literature in which PXR activation is the basis of observed drug-drug interactions, e.g., simultaneous administration of indinavir and St. John’s wort reduces exposure of the protease inhibitor (Piccitelli et al., 2000). Additionally, Ruschitzka et al. (2000) reported heart transplant rejections in patients concomitantly treated with cyclosporine and St. John’s wort. The authors report reduced oral bioavailability and increased hepatic clearance of the victim drug. Importantly, to interact with PXR and to activate transcription of the targeted genes, hyperforin has to enter the cell. So far, little is known about the transmembrane transport and cellular uptake of this molecule.

Recent findings by our group suggest that hyperforin is a substrate of OATP2B1, as was observed by testing whether the method of competitive counterflow (CCF) can be applied to identify substrates of this uptake transporter (Schäfer et al., 2018). OATP2B1 is a member of the organic anion-transporting polypeptide family, and facilitates the sodium-independent uptake of its substrates. Since its first description (Tamai et al., 2000) multiple endogenous and exogenous substrates of the transporter have been identified (Roth et al., 2012). OATP2B1 exhibits two substrate binding sites that can be distinguished in experimental setting by their contribution to the cellular uptake of estrone 3-sulfate (E1S); one accepts E1S at low concentration (binding site A), whereas the other (binding site B) mainly drives uptake at high concentrations of the sulfated steroid (Shirasaka et al., 2012). Another characteristic of OATP2B1 is its ubiquitous expression, with high amounts of the transporter in brain, heart, kidney, lung, mammary gland, placenta, platelets, skeletal muscle and skin (St-Pierre et al., 2002; Pizzagalli et al., 2003; Schiffer et al., 2003; Bronger et al., 2005; Grube et al., 2006; Niessen et al., 2009; Knauer et al., 2010; Sakamoto et al., 2013; Ferreira et al., 2018). Considering the abovementioned impact of PXR on hepatic clearance and oral bioavailability, it seems noteworthy that OATP2B1 is expressed in the sinusoidal membrane of hepatocytes (Kullak-Ublick et al., 2001) and in the intestine (Kobayashi et al., 2003; Keiser et al., 2017), thereby contributing to hepatic clearance and intestinal absorption, respectively. Even if several drug transporters are part of the PXR-regulated gene network, OATP2B1 is not (Knauer et al., 2013; Meyer zu Schwabedissen et al., 2018). However, our preliminary findings (Schäfer et al., 2018) suggest that drug-drug interactions involving hyperforin may not be limited to targets of PXR but may also involve OATP2B1-mediated uptake. It was the aim of the present study to further evaluate this notion.

Materials and Methods

Cell Culture. All cell lines were maintained at 37°C in a humidified atmosphere with 5% CO2. The cell lines Madin-Darby canine kidney epithelial cells (MDCKII; ATCC no. CRL-2936), HeLa (ATCC no. CCL2), HepG2 (ATCC no. HB-8065), and Caco-2 (ATCC no. HTB37) were originally obtained from the American Type Culture Collection (ATCC, Wesel, Germany) and were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich, Buchs, Switzerland) supplemented with 10% fetal calf serum (Sigma-Aldrich) and 1% stable glutamine (BioConcept, Basel, Switzerland). For Caco-2 cells the medium was supplemented with 1% penicillin/streptomycin (BioConcept, Basel, Switzerland). MDCKII-OATP2B1 cells have been established and characterized as described elsewhere (Grube et al., 2006) and were kept under continuous selection with 750 μg/ml hygromycin B (Carl Roth, Karlsruhe, Germany).

Transport Experiments. MDCKII-OATP2B1 cells were seeded in 24-well plates at a density of 50,000 cells/well (Eppendorf, Hamburg, Germany). After 1 day in culture, cells were treated with sodium butyrate (2 mM) and cultured for an additional day. Uptake experiments were started by washing the cells with prewarmed phosphate-buffered saline (PBS) followed by a 10-minute equilibration with Hanks’ balanced salt solution (HBSS, with sodium bicarbonate, without phenol red, pH 7.4, Sigma-Aldrich). To test the inhibitory potency of hyperforin (as hyperforin dicyclohexylammonium salt, Sigma-Aldrich) and hypericin (Toeris, Bio-Technne AG, Züri, Switzerland) cells were exposed to either estrone 3-sulfate (0.005 or 0.05 μM; Sigma-Aldrich) or 0.1 μM bromosulfophthalein (BSP; Sigma-Aldrich), supplemented with 50,000 dpm/well of [3H]-E1S (3 nM; Hartmann Analytic, Braunschweig, Germany), or [3H]-BSP (9 nM; Hartmann Analytic) in the presence of different concentrations of the respective compound. After 5 minutes of exposure the cells were washed with ice-cold PBS, lysed in 200 μl of 0.2% SDS-5 mM EDTA, and the cellular content of E1S or BSP was quantified determining the amount of tracer by liquid scintillation counting using the Rotiszint eco Plus (Carl Roth) and the Tri-Carb 2960TR counter (TopLab, Basel, Switzerland). An aliquot was used to assess the amount of protein in each well using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Reinch, Switzerland) and the microplate reader Infinite 200 Pro (Tecan, Männedorf, Switzerland). CCF experiments were performed as previously described by our group (Schäfer et al., 2018). MDCKII-OATP2B1 and MDCKII cells were seeded, treated, and prepared for transport experiments as described above. Since CCF experiments are conducted in the steady state, cells were preincubated with [3H]-E1S (100,000 dpm/well) for 30 minutes. For a time-dependent CCF experiment in MDCKII-OATP2B1 cells, the supernatant was exchanged to either [3H]-E1S alone or supplemented with hyperforin (5 μM) or hypericin (100 μM). Cellular accumulation of [3H]-E1S was measured as described above after 10, 20, 30, 45, 60, 90, 120, and 180 seconds. Since the system was equilibrated again after 90 seconds, this was defined as the time point when intracellular accumulation of [3H]-E1S was measured in CCF experiments. MDCKII-OATP2B1 and MDCKII cells were treated with [3H]-E1S until steady state before medium was changed to the same concentration of [3H]-E1S supplemented with hyperforin, hypericin, atorvastatin (2.5 μM; Sigma-Aldrich) as positive, and penicillin G (500 μM; Sigma-Aldrich) as negative control. For inhibition studies, 2.5 μM atorvastatin supplemented with 50,000 dpm/well [3H]-atorvastatin (5.7 nM; PerkinElmer, Waltham, MA) was used. Cellular accumulation after 5 minutes of exposure was assessed in presence of different concentrations of hyperforin (0.1, 1, 5 μM) or hypericin (10, 50, 100 μM).

Cell-Based Reporter Gene Assays. The previously reported CYP3A4-XREM-pGL3 plasmid was used to test the influence of OATP2B1 on PXR-mediated transactivation. Briefly, HepG2 and HeLa cells were seeded in 24-well plates at a density of 50,000 cells/well. The cells were then transfected with 250 ng of CYP3A4-XREM-pGL3 (Tirona et al., 2003), 25 ng of pRL-TK, 250 ng of PXR-XREM-pGL3 (Tirona et al., 2003), 250 ng of RXRα-pGL3, 25 ng of pRL-TK, 250 ng of OATP2B1-pGL3, or 250 ng of pEF6-V5/HIS as control (Promega, Dübendorf, Switzerland). An aliquot was used to assess the amount of protein in each well using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific), using 2.25 μg/μl of DNA jetPRIME (Polyplus distributed by Chemie Brunschwig, Basel, Switzerland). After 4 hours, the medium was changed, and the cells were kept in culture until treatment started 24 hours after transfection. Hyperforin, hypericin, bromosulfophthalein, and rifampicin were used at a final concentration of 0.1, 1, 10, and 10 μM, respectively. The formulations were tested at a 1/100 dilution of one tablet or capsule dissolved in 200 ml. To avoid phototoxicity the experiment was performed in the dark. Finally, the cells were lysed after 24 hours of exposure and luciferase activity was determined using the Dual-Luciferase Assay System (Promega, Dübendorf, Switzerland) and the plate reader Infinite M200 Pro (Tecan) as recommended by the manufacturer. The observed activity of the firefly luciferase was normalized to that of the Renilla.
Transwell Transport of Hyperforin and Hypericin. Experiments were performed as previously described (Meyer zu Schwabedissen et al., 2018). Briefly, Caco-2 cells were seeded at a density of 3 × 10^5 cells/well onto polycarbonate membranes with 0.4-μm pore size inserted in 12-well plates (Chemie Brunschwig, Basel, Switzerland) and cultivated for 14 days with medium change every second day. Measuring the transepithelial electrical resistance value of at least 200 Ω·cm² 1 day before the experiment confirmed the integrity of the monolayer. Furthermore, integrity was tested after the experiment by addition of 1 mg/ml Lucifer yellow (Sigma-Aldrich) to the apical and side measurement of absorbance in the basolateral compartment after 1 hour by spectrophotometry. On the day of the experiment, cells were washed once with prewarmed PBS before incubation with Krebs-Henseleit buffer (118 mM NaCl, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 11 mM glucose, 4.7 mM KCl) for 20 minutes at 37°C. The buffer was adjusted to a pH of 5.5 in the apical compartment and to a pH of 7.4 in the basolateral compartment. [³²P]-Atorvastatin (100,000 dpm/well) was added either to the apical or to the basolateral side to determine apical to basal (a→b) or basal to apical (b→a) permeability. Hyperforin (0.1 μM) was added to either the apical or the basal compartment. Amount of atorvastatin was assessed in 100-μl aliquots by scintillation counting as described above. The amount of [³²P]-atorvastatin per time was calculated as permeability coefficient (P_app) as previously described (Hubatsch et al., 2007). P_app of the apical (a→b) or of the b→a direction was used to calculate the uptake and efflux ratio, respectively, with the following equations:

\[
\text{uptake ratio } P_{\text{app}}(a \rightarrow b) = \frac{P_{\text{app}}(a \rightarrow b)}{P_{\text{app}}(b \rightarrow a)} \quad \text{efflux ratio } P_{\text{app}}(b \rightarrow a) = \frac{P_{\text{app}}(b \rightarrow a)}{P_{\text{app}}(a \rightarrow b)}
\]

Transport Studies with 11 Swiss Formulations Containing St. John’s Wort. Before transport experiments, pills were milled using the mixer mill MM 400 (Retsch GmbH, Haan, Germany) or capsules were opened to release the content. The concentration for inhibition experiments was estimated by calculating the expected concentration in the intestine when the pill or capsule would be ingested with 200 ml water. Two days before the experiment MDCKII-OATP2B1 cells were seeded as described above. After washing the cells with PBS and a 10-minute preincubation in Hanks’ balanced salt solution buffer, influence was tested by treating the cells with 0.005 μM aliquots by scintillation counting as described above. The amount of [³²P]-atorvastatin per time was calculated as permeability coefficient (P_app) as previously described (Hubatsch et al., 2007). P_app of the apical (a→b) or of the b→a direction was used to calculate the uptake and efflux ratio, respectively, with the following equations:

\[
\text{uptake ratio } P_{\text{app}}(a \rightarrow b) = \frac{P_{\text{app}}(a \rightarrow b)}{P_{\text{app}}(b \rightarrow a)} \quad \text{efflux ratio } P_{\text{app}}(b \rightarrow a) = \frac{P_{\text{app}}(b \rightarrow a)}{P_{\text{app}}(a \rightarrow b)}
\]

Statistical Analysis. Microsoft Excel (Microsoft, Redmond, USA) and GraphPad Prism software 6.0 (GraphPad Software, La Jolla, CA) was used to analyze the data sets reported herein. Tests used for statistical analysis are described in the context of data presentation. A P-value below 0.05 was considered statistically significant.

Results

Interaction of Hyperforin and Hypericin with OATP2B1-Mediated Uptake. To validate the interaction of hyperforin and hypericin with OATP2B1, their impact on the cellular accumulation of the known substrates estrone 3-sulfate (Pizzagalli et al., 2003) and bromosulphophthalein (Kullack-Ublick et al., 2001) was determined in MDCKII-OATP2B1 cells. As shown in Fig. 1 the estrone 3-sulfate inhibition studies accounted for the previously reported two binding sites (Shirasaka et al., 2012) testing the influence of hyperforin and hypericin on estrone 3-sulfate accumulation at low (Fig. 1, A and D; representing binding site A) and at high concentrations (Fig. 1, B and E, representing binding site B). For hyperforin, we observed concentration-dependent inhibition for both binding sites recognizing E₅S. The inhibition data were the basis for an estimation of the respective IC₅₀ values, which revealed the highest potency for binding site...
A-mediated transport of E1S, with an IC50 value of 0.32 μM (CI 0.24–0.42 μM, Fig. 1A), whereas for binding site B the IC50 value was 5.55 μM (CI 2.10–14.73 μM, Fig. 1B). Inhibition of OATP2B1-mediated cellular accumulation of BSP resulted in an estimated IC50 value for hyperforin of 0.82 μM (CI 0.53–1.26 μM, Fig. 1C). Similar results were obtained for hypericin, even if this compound exhibited much lower IC50 values for binding site A (20.79 μM; CI 13.90–31.10 μM, Fig. 1D), binding site B 256.2 μM (CI 119.4–550.0 μM, Fig. 1E), and for BSP 108.7 μM (CI 76.39–154.8 μM, Fig. 1F). To test whether hyperforin and hypericin are also substrates of OATP2B1 we applied the method of competitive counterflow. The basis of the experimental procedure used to identify substrates competing for substrate recognition is depicted in Fig. 1G. Briefly, interaction with the transporter is assessed in the steady state. Reduction of the intracellular estrone 3-sulfate amount in the equilibrium would be attributable to competitive inhibition, showing that the test compound is a substrate of the facilitating mechanism, whereas inhibitors would not influence the cellular equilibrium (Harper and Wright, 2013). As shown in Fig. 1H, presence of the known OATP2B1 substrate atorvastatin, as well as hyperforin (5 μM) or hypericin (100 μM), reduced the amount of E1S in the steady state (P < 0.05), thereby suggesting that both constituents of St. John’s wort are not only inhibitors but also substrates of OATP2B1. Penicillin G served as nonsubstrate control (Schäfer et al., 2018). In the CCF experiments in MDCKII cells, no influence on the cellular amount of E1S was observed for any of the compounds tested herein (Supplemental Fig. 1). Assessing the time-dependency of reaching the second equilibrium for hyperforin (Supplemental Fig. 2A) and hypericin (Supplemental Fig. 2B) revealed that it is reached after 90 seconds of exposure, which is comparable to that previously reported (Schäfer et al., 2018).

**Influence of OATP2B1 on Hyperforin-Mediated Transactivation of CYP3A4.** Hyperforin is a well known ligand and activator of the human pregnane X receptor, which thereby influences transcription of CYP3A4 (Moore et al., 2000). To verify that hyperforin is a substrate of OATP2B1, we deployed cell-based reporter gene assays to test the transactivation of a synthetic CYP3A4-XREM-pGL3 reporter gene construct. As shown in Fig. 2A, HepG2-cells transfected with PXR- and OATP2B1-mEF6 exhibited higher luciferase activation (P < 0.05) than cells not heterologously expressing the transporter. Testing the influence of 10 μM BSP on hyperforin-induced (0.1 μM) PXR-mediated transactivation of CYP3A4 in OATP2B1-overexpressing cells showed that presence of this competitive inhibitor of the transporter reduced luciferase activity statistically significantly. Importantly, BSP did not influence the luciferase activity (Fig. 2B).

**Impact of Hyperforin and Hypericin on Transcellular Fluxes in Caco-2 Cells.** Expression of OATP2B1 is assumed to contribute to intestinal absorption of orally applied drugs. Transcellular flux experiments using the intestinal model cell line Caco-2 cells are often applied in the preclinical phase to not only predict bioavailability but also to determine whether there is an active transport component influencing the transcellular path of a new molecular entity. OATP2B1 is expressed in Caco-2 cells and is part of the network of transporters influencing the transcellular flux (Meyer zu Schwabedissen et al., 2018). To determine whether hyperforin influences transcellular fluxes, and thereby oral bioavailability, experiments were performed using the known OATP2B1 substrate drug atorvastatin (Grube et al., 2006). At first, interaction of hyperforin with OATP2B1-mediated atorvastatin uptake was tested (Fig. 3A), revealing a decreased amount of intracellular atorvastatin with increasing concentrations (P < 0.05). As controls, BSP and E1S were included in the transport inhibition study. Estimation of the inhibitory potency, even though a limited number of data points was used as a basis, suggested an IC50 value for hyperforin of 0.23 μM (CI 0.11–0.48 μM). As shown in Fig. 3B, hyperforin increased the efflux ratio observed for atorvastatin (mean efflux ratio ± S.D.; hyperforin vs. DMSO: 2.863 ± 0.415 vs. 1.828 ± 0.332; P = 0.028). The observed effect can mainly be attributed to changes in the flux from apical (a) to basal (b), as there was a trend toward lower movement in this direction [mean Papp (a–b) ± S.D. (cm/s); hyperforin vs. DMSO: 1.33 × 10−6 ± 3.161 × 10−7 vs. 1.83 × 10−6 ± 2.621 × 10−7; P = 0.103, Fig. 3C)]. No trend for a change was observed for the atorvastatin flux in b to a direction [mean Papp (b–a) ± S.D. (cm/s); hyperforin vs. DMSO 3.81 ± 10−5 ± 1.153 × 10−5 vs. 3.36 × 10−5 ± 8.502 × 10−6; P = 0.615, Fig. 3D). Taken together these data suggest that hyperforin interacts with intestinal absorption of atorvastatin, which is in part mediated by OATP2B1.

**Effect of St. John’s Wort Formulations on OATP2B1-Mediated Transport.** St. John’s wort is widely used as medication to treat mild to moderate depression. In Switzerland there are currently 11 solid formulations marketed. These formulations contain different amounts of hypericin and hyperforin as reported by the manufacturer and summarized in Table 1. In a screening experiment, we tested for interaction of the formulations with OATP2B1-mediated E1S uptake in MDCKII-OATP2B1 cells. This screening was conducted using two concentrations of the respective formulation, representing a 1/100 or 1/1000 dilution of one tablet or capsule dissolved in 200 ml of liquid. As shown in Fig. 4A, none of the 1/1000 dilutions lowered the cellular accumulation of E1S compared with solvent control (P > 0.05). However, for the formulations Arkocaps, Deprivita, Hänsele Menopause, Hyperiplant, Jarsin, Sandoz Hypericum, and Solevita, we observed reduced OATP2B1-mediated uptake of estrone 3-sulfate for the 1/100 dilution (P < 0.05). Rebalance, Remotiv, Vogel Hyperimmed, and Vogel Hyperforce did not affect OATP2B1-mediated E1S uptake in our experiments.

Subsequently, we assessed the influence of the respective formulation on the PXR-mediated transactivation of CYP3A4 either in HepG2 (Fig. 4B), or HeLa (Fig. 4C). Notably, in accordance with our transport inhibition experiments, Rebalance, Remotiv, Vogel Hyperimmed, and Vogel Hyperforce did not affect OATP2B1-mediated E1S uptake in our experiments.

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Quantification of Hyperforin and Hypericin Content in the St. John’s Wort Formulations. As mentioned before, most manufacturers merely provide information on the content of hypericin in their formulation owing to the primal assumption that this component determines biologic activity of the extract. We quantified the amount of hyperforin (as the sum of hyperforin and adhyperforin) and hypericin (as the sum of hypericin and pseudohypericin) in each formulation by HPLC. As summarized in Table 1 and depicted in Supplemental Figs. 3 and 4, all formulations contained hypericin, but hyperforin was detected in all medications but Vogel Hyperimed and Vogel Hyperiforce and to a low extent in Rebalance and Remotiv. Importantly, these four medications neither interacted with OATP2B1-mediated E1S accumulation nor did they transactivate CYP3A4 in the cell-based
reporter gene assays reported herein, suggesting that hyperforin is the key determinant of the observed effects. To test this notion, we analyzed whether the relationship between hyperforin content and the observed luciferase activation assessed in transfected HepG2 (Fig. 5A) or HeLa cells (Fig. 5B) revealed a direct correlation. The association as determined by Pearson correlation was most pronounced when Hyperiplant, the formulation containing the highest amount of hyperforin, was excluded. An indirect and statistically significant correlation was observed for the percentage of E1S uptake and hyperforin content in MDCKII-OATP2B1 cells (Fig. 5C). Importantly, no correlation was observed for hypericin and the experimental results (Fig. 5, D–F).

**Discussion**

In this study we report that the constituents of St. John’s wort—hyperforin and to a lower extent hypericin—are inhibitors of the ubiquitously expressed membrane transporter OATP2B1. Furthermore, CCF experiments suggested that the compounds are not only inhibitors but also substrates of OATP2B1. This was further supported by findings in reporter gene assays, in which OATP2B1 enhanced hyperforin-induced transactivation of CYP3A4, which was reduced in the presence of the competitive inhibitor bromosulfophthalein. Hyperforin is known especially as a major determinant of drug-drug interactions observed during coadministration of St. John’s wort, with the expected result of a pronounced increase in CYP3A4 activity (Willson and Kliewer, 2002). However, the influence of concomitant use of St. John’s wort is not limited to CYP3A4-substrates as the underlying mechanism; it is the binding and activation of PXR that finally...
results in induced expression of multiple target genes, most of them with functions in drug elimination (Tolson and Wang, 2010). Indeed, one compound exemplifying this is the cardiac glycoside digoxin (Johne et al., 1999). This drug is commonly used in clinics and is not a CYP3A4 substrate (Lacarelle et al., 2010). Specifically for ABCB1, some data suggest that hyperforin may influence transport activity directly (Hennessy et al., 2002). Our data suggest that, when present, hyperforin acutely interacts with oral absorption of OATP2B1 substrates, enhancing the complexity of drug-hyperforin interactions that should be considered when evaluating the interaction potential of St. John’s wort preparations.

In addition to its involvement in intestinal drug absorption, OATP2B1 is assumed to contribute to the hepatocellular uptake. Recent findings by Lutz et al. (2018) suggest that there is at least some induction of OATP-mediated transport, as observed for concomitant treatment with rosuvastatin and pravastatin and increasing doses of rifampin. Even if OATP2B1 (OATP-B) may contribute to the hepatocellular uptake of these statins (Knauer et al., 2010; Shirasaka et al., 2010), OATP2B1 is not regulated in primary human hepatocytes treated with the PXR-inducer (Jigorel et al., 2006). Although there was no direct regulation by PXR, we recently reported interaction of the transporter with PDZ domain containing 1 (PDZK1), which influences OATP2B1 membrane localization (Ferreira et al., 2018). PDZK1 is transcriptionally controlled by multiple nuclear receptors (Ferreira et al., 2018; Prestin et al., 2017). Accordingly, whether long-term exposure to hyperforin influences localization of OATP2B1 remains to be determined, as we only tested the acute influence. Our findings suggest that the intracellular abundance of hyperforin, and thereby PXR-mediated transactivation of CYP3A4, is influenced by OATP2B1. Considering this finding, one might speculate that changes in OATP2B1 activity could influence the extent of PXR activation. Multiple mechanisms affect the activity of a transporter, and one possibility is the

### TABLE 1

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<th>Formulation (Tradename)</th>
<th>Percent Hypericin (mg/100 mg)</th>
<th>Percent Hyperforin (mg/100 mg)</th>
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<tr>
<td>VOGEL HYPERIMED®</td>
<td>0.5-0.8</td>
<td>0.08</td>
<td>± 0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>VOGEL HYPERIFORCE®</td>
<td>0.2-2.1</td>
<td>0.08</td>
<td>± 0.01</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*The content of hypericin corresponds to the sum of the content of hypericin and pseudohypericin.

*The content of hyperforin corresponds to the sum of the content of hyperforin and pseudohyperforin.
change in expression by transcriptional modifiers. Another would be modifications in genetic information with influence on transport activity. We recently reported that OATP2B1 is transcriptionally modulated by thyroid hormones (Meyer zu Schwabedissen et al., 2018), thereby associating thyroid hormone status to OATP2B1 transport function, and Nozawa et al. (2002) identified genetic polymorphisms that changed the maximal transport velocity ($V_{\text{max}}$) of OATP2B1.

Testing extracts from all oral formulations of St. John's wort currently marketed in Switzerland for their influence on OATP2B1 transport activity, and the influence of OATP2B1 on their transactivating activity, revealed a clear association
between hyperforin content and the observed effect for most formulations. Importantly, no such association was observed for hypericin content and PXR-mediated transactivation, thus confirming that hypericin is no activator of PXR (Moore et al., 2000; Wentworth et al., 2000). Furthermore, there is no clear association of hypericin content and OATP2B1 inhibition, which is in line with the much lower inhibitory potency observed for hypericin compared with hyperforin. This indicates that hyperforin is the component of St. John’s wort extracts involved in interaction with OATP2B1-mediated uptake.

The formulations were tested in cell-based reporter gene assays using HepG2 or HeLa cells as cellular models. In the liver cell model we observed a huge induction of luciferase activity of about 50- to 100-fold, whereas the increase in activity was only about 5- to 7-fold in HeLa cells, with some formulations not affecting transactivation. On the one hand, the difference in extent of activation might certainly be explained by endogenous expression of HNF4α in HepG2 cells, which is not present in HeLa cells as confirmed by Western blot analysis in this study and previously reported by Knauer et al. (2013). Importantly, HNF4α is permissive for the transcriptional regulation by PXR (Ma et al., 2008). On the other hand, the fact that we observed induction in HeLa cells and a clear association of the extent of activation with the hyperforin content suggests that hyperforin is the driving component, but also that there is at least some HNF4α-independent PXR activation.

As mentioned before, the hyperforin content was directly correlated with the observed luciferase activation. However, this association was most pronounced when Hyperiplant was excluded from the correlation. In other words, the observed effect of Hyperiplant on OATP2B1-mediated transport or CYP3A4 activation was lower than expected considering the hyperforin content. In this context, it seems noteworthy that hyperforin is phototoxic (Onoue et al., 2011), inducing acute cell lysis. Even though our experiments were conducted under light protection, we cannot exclude the presence of some toxicity that could have modified the experimental outcome.

Taken together we report that hyperforin is a potent inhibitor of OATP2B1-mediated cellular uptake and we suggest that it is also a substrate. Inhibition of the transporter may influence the contribution of OATP2B1 to pharmacokinetics of its substrate drugs. Moreover, OATP2B1 activity influences intracellular accumulation of hyperforin and thereby its binding to the xenosensor. With this study, we added to the complexity of potential mechanisms that should be included in the evaluation of potential drug-drug interactions associated with the dispensing of St. John’s wort.

Acknowledgments

The study reported herein will be part of the thesis of A.M.S.

Authorship Contributions

Participated in research design: Schäfer, Potterat, Meyer zu Schwabedissen.
Conducted experiments: Schäfer, Potterat, Seibert, Fertig, Meyer zu Schwabedissen.
Contributed new reagents or analytic tools: Potterat, Meyer zu Schwabedissen.

Figure 5. Association of hyperforin or hypericin content with the observed effects in cell-based reporter gene assays, or transport inhibition studies. Luciferase activity was determined in transiently transfected HepG2 (A, D) or HeLa cells (B, E). Inhibition studies were conducted in MDCKII stably expressing OATP2B1 (C, F). Association of the observed effect with hyperforin or hypericin content was analyzed by linear regression (indicated by dotted line, shown with confidence interval), correlation was determined by calculating the Pearson coefficient. Data are presented as mean ± S.D. with results of n = 3 independent experiments performed in biologic triplicates. R, Pearson coefficient; open symbol indicated “Hyperiplant”, which was neither included in the linear regression nor the calculation of the Pearson coefficient.
Perfomed analysis data: Schäfer, Potterat, Meyer zu Schwabedissen.
Wrote or contributed to the writing of the manuscript: Schäfer, Potterat, Seibert, Fertig, Meyer zu Schwabedissen.

References


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