PXR/CYP3A4-Humanized Mice for Studying Drug–Drug Interactions Involving Intestinal P-Glycoprotein

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ABSTRACT: Rodent models are less suitable for predicting drug–drug interactions at the level of the human intestinal mucosa, especially when nuclear receptors such as pregnane X receptor (PXR) are involved. Recently, a transgenic mouse model, expressing both human PXR and CYP3A4, was developed and shown to be a better predictor of CYP3A4 induction by xenobiotics in humans as compared to wild-type mice. In the present study, we tested the hypothesis that this mouse model can also predict PXR-mediated induction of intestinal P-gp in humans. By use of the in situ intestinal perfusion technique with mesenteric blood sampling, the effect of oral rifampicin treatment on intestinal permeability for the HIV protease inhibitor darunavir, a dual CYP3A4/P-gp substrate, was investigated. Rifampicin treatment lowered the intestinal permeability for darunavir by 50% compared to that in nontreated mice. The P-gp inhibitor GF120918 increased the permeability for darunavir by 400% in rifampicin-treated mice, whereas this was only 56% in mice that were not treated, thus indicating P-gp induction by rifampicin. The nonspecific P450 inhibitor aminobenzotriazole (100 μM) did not affect the permeability for darunavir. Quantitative Western blot analysis of the intestinal tissue showed that rifampicin treatment induced intestinal P-gp levels 4-fold, while CYP3A4 levels remained unchanged. Oral co-administration of rifampicin with the phytochemical sulforaphane for 3 days increased the permeability for darunavir by 50% compared to that with rifampicin treatment alone. These data show that PXR/CYP3A4-humanized mice can be used to study the inducing effects of xenobiotics on intestinal P-gp.

KEYWORDS: in situ intestinal perfusion, humanized mouse, PXR, P-glycoprotein, rifampicin, darunavir

INTRODUCTION

In order to investigate intestinal drug absorption, several techniques are currently being used, including cell culture models, animal intestinal tissue, and in vivo experiments. Drug transport across Caco-2 cells (only for passively transported drugs)1 and rodent intestinal tissue2,3 provides a reliable indication of the fraction absorbed in humans. Unfortunately, these models are less suitable for predicting drug–drug interactions at the level of the human intestinal mucosa, especially when nuclear receptors are involved. For example, the pregnane X receptor (PXR), a xenobiotic receptor, is not expressed in Caco-2 cells.4 PXR activation results in upregulation of many P450s, including CYP3A4, CYP3A5, CYP3A7, and the drug transporter P-glycoprotein (P-gp). Human and mouse PXR exhibit different activation profiles in response to xenobiotics,5 thus explaining why rodents have limited predictive value with respect to nuclear receptor-mediated drug–drug interactions in humans. For example, rifampicin is a strong activator of human PXR but is only a poor activator of rodent PXR. Conversely, rat and mouse PXR are activated by pregnenolone 16α-carbonitrile, which does not activate human PXR. Because of the limitations of both Caco-2 cells and rodent intestinal tissue, a better model is required for predicting intestinal drug–drug interactions in humans.

Rifampicin is currently being used as a first-line drug in the treatment of active tuberculosis. Unfortunately, it is often the cause of drug–drug interactions as it induces many P450s and drug transporters,6 thereby potentially limiting drug bioavailability. For example, the oral bioavailability of the P-gp substrate digoxin was shown to be significantly lower when co-administered with rifampicin, which can be explained by P-gp induction at the level of the intestine.7 Furthermore, HIV/TB patients receiving rifampicin should avoid HIV protease inhibitor (PI)-based regimens because rifampicin affects the oral bioavailability of PIs, which are substrates of CYP3A4 and P-gp, resulting in subtherapeutic plasma concentrations.

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Darunavir is a second generation PI with antiviral efficacy against HIV-1 with multiple resistance mutations to PIs.\(^6\) Using the in situ intestinal perfusion technique with mesenteric blood sampling\(^6\) in P-gp knockout and NMRI mice, P-gp was found to significantly limit the intestinal permeability for darunavir.\(^10,11\) In these mice, P-gp-mediated darunavir transport was inhibited by co-perfusion with the “pharmacokinetic booster” rifampicin, which is generally co-administered with PIs in order to increase their plasma levels.

A transgenic mouse model, expressing both human PXR and CYP3A4, was developed that can serve as a useful tool to study the effect of xenobiotics on the expression of CYP3A4 in humans.\(^12\) We tested the hypothesis that this mouse model can also be used for predicting the induction of intestinal P-gp by xenobiotics in humans. By performing the in situ intestinal perfusion technique in these PXR/CYP3A4-humanized mice, the effect of oral rifampicin treatment on the intestinal absorption of the PI darunavir was determined, thereby determining the relative contributions of both CYP3A4 and P-gp (mdr1a/1b). The effect of rifampicin treatment on the intestinal CYP3A4 and P-gp protein levels was determined by Western blot analysis.

The phytochemical sulforaphane is formed after hydrolysis of glucoraphanin in many cruciferous vegetables (including broccoli and cabbage).\(^13\) Sulforaphane has previously been shown to inhibit CYP3A4 and P-gp induction after rifampicin treatment in primary human hepatocytes, thereby inhibiting PXR-mediated induction of drug clearance,\(^14\) which would suggest its use to reduce PXR-mediated drug–drug interactions. However, experimental data regarding the use of sulforaphane appear to be inconclusive. In a recent clinical study in humans where rifampicin treatment decreased midazolam AUC by 70%, concomitant administration of sulforaphane did not reduce the effect of rifampicin.\(^15\) These investigators suggested that the hepatic sulforaphane concentrations were probably too low to inhibit PXR activation. In the current study, we tested the hypothesis that sulforaphane, which is present at relatively higher concentrations in the intraluminal environment, is able to inhibit a possible effect of rifampicin treatment on the uptake of darunavir in the intestine of the PXR/CYP3A4-humanized mouse.

### MATERIALS AND METHODS

**Chemicals.** Darunavir ethanolate was provided by the NIH AIDS Research and Reference Reagent Program (Germantown, MD). R426857 was kindly donated by Johnson & Johnson (Beerse, Belgium). Rifadine (rifampicin) was from Sanofi Aventis (Diegem, Belgium). GF120918 (elacridar) was provided by GSK (London, U.K.). Ketamine (Anesketin) and xylazine (Xyl-M 2%) were from Eurovet (Heusden, Belgium) and VMD (Arendonk, Belgium), respectively. The protease inhibitor cocktail for use with mammalian cell extracts and albumin from bovine serum (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium acetate trihydrate and methanol were purchased from VWR (Linderode, Germany). Sodium taurocholic acid practical grade was purchased from MP Biomedicals (Illkirch Cedex, France). Phospholipon 90G (lecithin) was from Nattermann Phospholipid GmbH (Köln, Germany). All other reagents were used as supplied. Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, U.K.). Stock solutions of rifampicin and sulforaphane were prepared in DMSO.

**Animals.** Transgenic mice expressing human PXR and CYP3A4 (TgCYP3A4/hPXR) were transferred from the National Cancer Institute (Bethesda, MD) and housed at KU Leuven (Leuven, Belgium). NMRI (Naval Medical Research Institute) mice were purchased from Janvier (Le Genest Saint Isle, France). Only male mice were used in the experiments. In the rifampicin-treated group, mice were doused by oral gavage with 10 mg/kg of rifampicin dissolved in PBS during the 3 days prior to the day of the experiment. Sulforaphane was orally administered at 5 mg/kg during the 3 days together with rifampicin. The molar ratio of sulforaphane to rifampicin was 2.3 to 1. Approval for the mouse experiments was granted by the Institutional Ethical Committee for Animal Experimentation of the KU Leuven.

**Media.** Fasted state simulated intestinal fluid (FaSSIF) was made according to the composition reported by Vertzoni et al. (revised standard FaSSIF with practical grade taurocholate and soybean lecithin).\(^16\)

**In Situ Intestinal Perfusion.** The setup for the in situ perfusion experiments in mice has previously been described by Mols et al.\(^9\) The perfusion experiments were performed using an open-loop setup. A segment of the ileum (between 2 and 3 cm) was perfused at a flow rate of 0.2 mL/min. The exact length of the segment was taken into account when calculating the \(P_{app}\) value (see section Permeability Calculations). The perfusate consisted of FaSSIF containing darunavir (100 \(\mu M\)) in the absence or presence of GF120918, a specific P-gp inhibitor at 4 \(\mu M\),\(^57\) and in the absence or presence of aminobenzotriazole (100 \(\mu M\)), a nonspecific P450 inhibitor. FaSSIF, which contains bile salts and lecithin, was chosen as solvent system to simulate biorelevant conditions. This is important because it was previously shown that compounds present in intestinal fluids may alter the functionality of drug transporters.\(^18\) Blood was collected continuously from the mesenteric vein for 60 min at 5-min time intervals. After the experiment, the intestine was removed, flushed with ice cold PBS, and stored at −30 °C until further use.

**Tissue Homogenization and Western Blot Analysis.** Isolation of enterocytes was performed based on the method previously described by Mohri and Uesawa.\(^19\) All solutions were used at 4 °C. The frozen intestines were thawed in ice-cold PBS. One end was clamped and the intestine was filled with solution A (PBS, pH 7.2, containing 5 mM EDTA, 0.5 mM dithiothreitol, 5 U/mL heparin, and protease inhibitor cocktail (1%)). The intestine was tapped gently several times during 10 min, after which the solution was collected and kept on ice. This procedure was repeated four times. The collected cells were centrifuged at 800 \(\times g\) for 10 min at 4 °C and resuspended in solution B (pH 7.8, containing 10 mM HEPES, 250 mM sucrose, 25 mM KCl, 1 mM EDTA, and protease inhibitor cocktail (1%)). Next, the cells were added to the glass tube of a Potter-Elvehjem homogenizer (VWR International) and homogenized on ice by 10 strokes with a PTFE pestle at rotating high speed. Protein contents were determined with the method of Lowry\(^20\) using BSA as standard. For Western blot analysis, 30 \(\mu g\) of protein from each sample was loaded onto 10% SDS-polyacrylamide gels. The following primary antibodies were used: P-gp [H-241, rabbit polyclonal antibody, Santa Cruz Biotechnology (Heidelberg, Germany)], human CYP3A4 [ab22704, rabbit polyclonal antibody, Abcam (Cambridge, England)].
U.K.), and β-actin [ab8226, mouse monoclonal antibody, Abcam]. Detection was performed using HRP-labeled secondary antibodies [goat polyclonal anti-rabbit immunoglobulins (P0448, Dako (Heverlee, Belgium)] and the Amersham ECL plus detection kit from GE Healthcare (Diegem, Belgium). Western blots were quantified using the ImageJ 1.45 software (NIH) after scanning of the films.

**Analysis of the Blood Samples.** Quantifying darunavir or R426857 in the blood samples required extraction: 100 μL of blood was diluted into 400 μL of a mixture of KH2PO4 (0.1 M, pH 6.0) and methanol (80:20 v/v); subsequently, 100 μL of internal standard solution (butyl-4-hydroxybenzoate, 10 μg/mL) was added. After addition of 4 mL of diethyl ether, 1 min of shaking, and centrifugation (2880 × g, 5 min), the organic layer was transferred to a clean test tube and evaporated to dryness under a gentle stream of air. The residue was dissolved in 150 μL of a solution of water and methanol (50:50 v/v), of which 10 μL was injected into the HPLC system. Darunavir and R426857 were detected with a fluorescence detector. The HPLC system consisted of a Waters Alliance 2695 separations module and a Nova-Pak C18 Radial-Pak (4 mm, 8 mm) column under radial compression (Waters, Milford, MA). Fluorescence (excitation 268 nm, emission 347 nm) was monitored by a Waters fluorescence detector (W2475). The mobile phase consisted of 25 mM sodium acetate (pH 5.5) and methanol (40:60 v/v); the flow rate amounted to 1.3 mL/min. The retention times of darunavir and the internal standard were 20 μM. The assessment of interday repeatability of calibration curve was linear over the concentration range of 20–20 μM. The deviation from the theoretical concentration amounted to −4.6%.

**Permeability Calculations.** By measuring the amount of darunavir in the mesenteric blood, the apparent permeability (P_{app}) was determined, which reflects the overall intestinal absorption process including passive diffusion, the contribution of transporters, as well as intestinal metabolism. P_{app} values were calculated from the cumulative amount of darunavir appearing in the blood between 40 and 60 min according to the following equation:

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot AC_{donor}}$$

where Q is the cumulative amount of drug appearing in the mesenteric blood, A is the surface area of the perfused cylindrical intestinal segment, and C_{donor} is the drug concentration in the perfusate. The surface area was calculated with the following equation:

$$A = 2\pi rl$$

where r is the radius of the intestinal segment (1 mm for the mouse ileum) and l is the length of the perfused cylindrical intestinal segment.

**Statistics.** Statistical analysis was performed using an unpaired t test or one-way ANOVA followed by Dunnett’s test, as specified in the legends of the figures. P-values of less than 0.05 are considered as statistically significant.

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**RESULTS**

**System Suitability.** A mouse intestinal perfusion model with mesenteric blood sampling using a closed-loop setup has been previously described. Recently, we reported the use of an open-loop setup, which has the advantage that a constant C_{donor} was being maintained during the entire experiment (C_{donor} was measured at the beginning and the end of each experiment). The cumulative transport of darunavir, corrected for the length of the perfused intestinal segment, is shown as a function of time as the mean of 3 individual experiments in NMRI mice (Figure 1). During transport experiments, the steady-state darunavir transport was typically reached before 30 min, after which the transport rate was fairly constant.

![Figure 1](Image 360x471 to 529x589)

*Figure 1. Transepithelial transport of darunavir as a function of time using the mouse in situ intestinal perfusion model. The ileum of NMRI mice was perfused with FaSSIF containing darunavir (100 μM). Cumulative transport values, corrected for the length of the intestinal segment, are shown as the mean ± SD (n = 3).*

**Effect of Rifampicin on the Intestinal Absorption of Darunavir in NMRI Mice.** In the first set of experiments, the effect of oral rifampicin treatment on the intestinal transport of darunavir in NMRI mice was determined. NMRI mice were orally treated with rifampicin at pharmacological doses (10 mg/kg) for 3 days prior to the experiment. On the fourth day, we performed in situ intestinal perfusion studies with mesenteric blood sampling where the perfusate consisted of darunavir (100 μM) dissolved in FaSSIF. As a result of oral rifampicin treatment, a 66% increase in intestinal permeability was observed for darunavir that, however, did not reach statistical significance (Figure 2).

**Effect of Rifampicin on the Intestinal Absorption of Darunavir in TgCYP3A4/hPXMR Mice.** We tested the hypothesis that the TgCYP3A4/hPXMR mouse model is suitable to study the induction of intestinal P-gp by rifampicin at pharmacological doses. We first investigated whether this transporter has a modulatory effect on the intestinal permeability for darunavir in these mice. When the intestine of the humanized mice (which had not been treated with rifampicin) was perfused with darunavir and the P-gp inhibitor GF120918 (4 μM), the intestinal permeability for darunavir was increased by 56% (p < 0.05), indicating that P-gp limits the intestinal permeability for darunavir (Figure 3). Next, we tested the hypothesis that rifampicin treatment affects the intestinal absorption of darunavir. Oral dosing of rifampicin (10 mg/kg) for 3 days prior to the intestinal perfusion experiment caused a decrease of 50% in the intestinal permeability for darunavir compared to that in untreated mice. This observation may be explained by an increased biochemical barrier function of the intestinal mucosa, either by an induction of intestinal CYP3A4,
di using one-way ANOVA followed by Dunnett ± mean evaluated using an unpaired t test. Conditions in the group that was not treated with rifampicin was statistically different from control condition (p < 0.05). Statistical significance between the different conditions was evaluated using an unpaired t test. Control condition was not statistically different from rifampicin-treated condition.

Figure 2. Apparent permeability values of the ileum of NMRI mice for darunavir (100 μM) using FaSSIF as perfusate medium in mice that were not treated with rifampicin (control) and mice orally treated with rifampicin (10 mg/kg) for 3 days. Bars represent the mean ± SD (n = 3). Statistical significance between the control and rifampicin-treated mice was evaluated using an unpaired t test. Control condition was not statistically different from rifampicin-treated condition.

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Figure 3. Apparent permeability values of the ileum of TgCYP3A4/hPXR mice for darunavir (100 μM) using FaSSIF as perfusate medium. Experiments were performed in mice that were not treated with rifampicin (open bars) and mice orally treated with rifampicin (10 mg/kg) for 3 days (gray bars), in the absence and presence of the P-gp inhibitor GF120918 (GF, 4 μM) or the nonspecific P450 inhibitor aminobenzotriazole (ABT, 100 μM). Bars represent the mean ± SD (n = 3). Statistical significance between the different conditions in the group that was not treated with rifampicin was evaluated using an unpaired t test. (+) Significantly different from control condition (p < 0.05). Statistical significance between the different conditions in the rifampicin-treated group was evaluated using one-way ANOVA followed by Dunnett’s test. (†) Significantly different from control condition (p < 0.05).

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P-gp, or both. To investigate whether intestinal P-gp expression was induced by rifampicin treatment, GF120918 (4 μM) was added to the intestinal perfusate, which increased the intestinal permeability for darunavir by 400% (p < 0.05). When we compared the effect of GF120918 in the untreated mice with the rifampicin-treated mice, there was a strong indication of P-gp induction. To investigate whether intestinal CYP3A4 expression was induced by rifampicin treatment, the nonspecific P450 inhibitor aminobenzotriazole (100 μM) was included in the perfusate. The use of aminobenzotriazole in rifampicin-treated mice did not change the intestinal permeability for darunavir, thus suggesting that rifampicin treatment has no effect on the intestinal CYP3A4 expression or functionality.

Intestinal Metabolism of Darunavir in TgCYP3A4/hPXR Mice. Darunavir is mainly metabolized by CYP3A4 in humans, with R426857 being the most prominent metabolite present in the plasma. To investigate whether intestinal metabolism limits the absorption of darunavir, the mesentric blood that was collected during the in situ intestinal perfusion experiments was analyzed for the presence of R426857. Indeed, the intestinal metabolism of darunavir was very low in the TgCYP3A4/hPXR mice, where the concentration of R426857 was less than 0.8% of this of darunavir. The relative amount of R426857 to darunavir was higher in the mice that were perfused with GF120918, being 1.6% and 3.1% for the untreated and rifampicin-treated mice, respectively.

Effect of Rifampicin on the Intestinal Protein Expression Levels of CYP3A4 and P-gp in TgCYP3A4/hPXR Mice. Western blot analysis of the intestinal tissue of the TgCYP3A4/hPXR mice was performed to investigate the effect of rifampicin treatment on CYP3A4 and P-gp protein levels. Rifampicin treatment did not increase the intestinal expression levels of CYP3A4, while P-gp expression was increased 4-fold (Figure 4). Since the TgCYP3A4/hPXR mice also express murine Cyp3a isoforms in addition to CYP3A4, we investigated whether there was cross reactivity of the CYP3A4 antibodies with rodent Cyp3a. Using rat intestinal and liver homogenate, we found that the CYP3A4 antibodies did not react with rat Cyp3a, which corresponded with the manufacturer’s data (data not shown).

Effect of Sulforaphane on the Intestinal Absorption of Darunavir. We tested the hypothesis that the PXR antagonist sulforaphane can neutralize the limiting effect of rifampicin on the intestinal absorption of darunavir. Oral co-administration of sulforaphane (5 mg/kg) with rifampicin (10 mg/kg) for 3 days prior to the intestinal perfusion experiments resulted in a 50% increase in permeability for darunavir compared to that in mice treated with rifampicin alone (Figure 5). Although this difference was not statistically significant, these data suggest that the limiting effect of rifampicin on the intestinal absorption...
permeability for darunavir compared to that in TgCYP3A4/hPXR mice that were not treated with rifampicin. Because this observation may be attributed to an induction of intestinal CYP3A4, P-gp, or both, we further investigated each hypothesis. In rifampicin-treated TgCYP3A4/hPXR mice, perfusion with the P-gp inhibitor GF120918 caused an increase in darunavir permeability of 400%, while in the nontreated mice, GF120918 only increased darunavir permeability by 56%. These data are consistent with rifampicin-mediated induction of intestinal P-gp. Furthermore, it was remarkable that GF120918 tended to increase the apparent permeability for darunavir to a higher level in the rifampicin-treated mice ($P_{app} = 61.3 \pm 9.9 \times 10^{-6}$ cm/s) as compared to that in the nontreated mice ($P_{app} = 45.7 \pm 6.3 \times 10^{-6}$ cm/s). This difference was however not statistically significant. Use of the nonspecific P450 inhibitor aminobenzotriazole had no impact on darunavir permeability, thus suggesting no effect of rifampicin on intestinal CYP3A4 functionality in this mouse model. Western blot analysis of the intestinal tissues confirmed our observations obtained from the in situ intestinal perfusion experiments: P-gp expression levels had increased 4-fold after rifampicin treatment, while CYP3A4 expression levels remained unchanged.

Since the TgCYP3A4/hPXR mice were not humanized for P-gp (MDR1), activation of human PXR appears to induce the expression of mouse P-gp in the brain of transgenic hPXR mice.43 Comparing our data with the literature, an induction of intestinal CYP3A4 protein levels was observed after rifampicin treatment,12 however, in this study rifampicin was administered for 6 days at a dose of 100 mg/kg, which was 10 times higher than the dose we applied. Another study in humans showed a significant increase in intestinal CYP3A4 mRNA and protein levels after 10 days of oral rifampicin administration at a dose of 600 mg/day.24 The reason why we did not observe any induction of CYP3A4 remains unclear; however, literature data suggest that CYP3A4 is highly constitutively expressed in the intestine of TgCYP3A4/hPXR mice, and thus the extent of induction is generally lower compared to robust CYP3A4 induction in the liver, where the constitutive expression of CYP3A4 is lower.25 After analyzing the mesenteric blood for the most prominent metabolite of darunavir present in human plasma (R426857), intestinal darunavir metabolism appeared to be very low. This was surprising, since intestinal CYP3A4 protein levels were readily detectable. Nevertheless, it is possible that darunavir inhibits its own intestinal metabolism since it was found to be an inhibitor of CYP3A4 with an inhibitory constant ($K_i$) value of 0.40 μmol/L, which is well within the range of clinically relevant concentrations.26 The darunavir concentrations at the level of the intestine are expected to be even higher. Also, the much lower intestinal P450 levels relative to the liver may further contribute to an inhibition of the available enzymes. Using human intestinal microsomes, we found that darunavir (5 μM) partially inhibits the metabolism of lopinavir (5 μM), while darunavir itself was not metabolized, indicating that darunavir inhibits CYP3A4-mediated metabolism (data not shown). The amount of darunavir metabolites measured in the mesenteric blood was highest when using the P-gp inhibitor GF120918, suggesting that most of the darunavir metabolites that are formed in the enterocytes are effluxed into the intestinal lumen by P-gp instead of being taken up into the blood via the basolateral membrane. This could however, not be verified experimentally.

**Discussion**

The aim of this study was to evaluate whether TgCYP3A4/hPXR mice can be used to predict the effect of xenobiotics on the intestinal expression levels of P-gp in humans. The use of wild-type mice in nuclear receptor-mediated drug–drug interaction studies may have only limited predictive value toward the human situation, because significant species differences exist in the ligand binding domain of PXR.5 In order to circumvent this problem, a transgenic mouse containing the human PXR gene (on the Pxr-null background), along with the CYP3A4 and CYP3A7 genes (TgCYP3A4/hPXR mice), was generated and used to demonstrate rifampicin–PI interactions, which could not be seen in mice without human PXR.12 Besides inducing many of the P450 enzymes in humans, oral rifampicin treatment was also shown to increase intestinal P-gp expression levels.7 The current study has investigated whether the TgCYP3A4/hPXR mouse model allows us to make the same observations. Using the in situ intestinal perfusion technique with mesenteric blood sampling, the effect of rifampicin treatment on the intestinal absorption of the PI darunavir was investigated. Rifampicin was orally administered at a dose of 10 mg/kg, which corresponds to the recommended dose in humans (600 mg per day). Darunavir was chosen as model compound because it is a substrate of both CYP3A4 and P-gp, which allows us to investigate the effect of rifampicin treatment at the level of both drug transporters and metabolism.

In a first set of experiments, the standard NMRI mice containing mouse PXR were used to investigate the effect of oral rifampicin treatment on the intestinal permeability for darunavir. As expected, rifampicin did not decrease the intestinal permeability for darunavir because rifampicin is not an activator of mouse PXR. Instead, rifampicin caused a 66% increase in darunavir permeability, which may be explained by an inhibition of P-gp by rifampicin12 still present in the enterocytes.

In a second set of experiments, TgCYP3A4/hPXR mice were dosed with rifampicin, after which a 50% decrease was observed in the intestinal permeability for darunavir compared to that in TgCYP3A4/hPXR mice that were not treated with rifampicin.
since we did not measure the appearance of metabolites in the perfusate.

In a final set of experiments, the effect of the phytochemical sulforaphane, a PXR antagonist, on the drug–drug interaction between rifampicin and darunavir at the level of the intestine was investigated. Although sulforaphane was previously shown to reduce CYP3A4 and P-gp induction by rifampicin in primary human hepatocytes, a recent clinical trial showed that sulforaphane was unable to reduce CYP3A4 induction by rifampicin in humans. It should be noted that the sulforaphane levels in the liver were probably too low to inhibit PXR activation by rifampicin. We found that co-administration of sulforaphane (5 mg/kg) with rifampicin (10 mg/kg) for 3 days prior to our experiments increased the intestinal permeability for darunavir by 50% compared to that with rifampicin treatment alone. This indicates that, for intestinal permeability for darunavir by 50% compared to that with rifampicin treatment alone. This indicates that, for compounds that are a substrate for P-gp and for which first-pass elimination is not primarily mediated by hepatic metabolism, sulforaphane may (partially) inhibit the effects of rifampicin on PXR. It also needs to be mentioned that the metabolism, sulforaphane may (partially) inhibit the effect in the liver in view of the much higher local sulforaphane concentrations in the intestine.

## CONCLUSION

We conclude that, in TgCYP3A4/hPXR mice, oral rifampicin treatment significantly decreased the intestinal permeability for darunavir by induction of P-gp; CYP3A4 protein levels remained unchanged. Because this could not be observed in conventional mice, PXR/CYP3A4-humanized mice are a promising tool to study PXR/P-gp-mediated drug–drug interactions at the level of the intestine.

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Notes
The authors declare no competing financial interest.

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## ABBREVIATIONS

PXr, pregnane X receptor; P450, cytochrome P450; FaSSIF, fasted state simulated intestinal fluid; PBS, phosphate buffered saline; \( P_{\text{app}} \), apparent permeability coefficient; P-gp, P-glycoprotein; PI, HIV protease inhibitor; GF120918, N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isouquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide

## REFERENCES


