

Amendment history:

- [Corrigendum](#) (August 2002)

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J Clin Invest. 1999;104(2):147-153. <https://doi.org/10.1172/JCI6663>.

Article

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The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin

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Received for publication February 26, 1999, and accepted in revised form June 3, 1999.

Recent data point to the contribution of P-glycoprotein (P-gp) to digoxin elimination. On the basis of clinical observations of patients in whom digoxin levels decreased considerably when treated with rifampin, we hypothesized that concomitant rifampin therapy may affect digoxin disposition in humans by induction of P-gp. We compared single-dose (1 mg oral and 1 mg intravenous) pharmacokinetics of digoxin before and after coadministration of rifampin (600 mg/d for 10 days) in 8 healthy volunteers. Duodenal biopsies were obtained from each volunteer before and after administration of rifampin. The area under the plasma concentration time curve (AUC) of oral digoxin was significantly lower during rifampin treatment; the effect was less pronounced after intravenous administration of digoxin. Renal clearance and half-life of digoxin were not altered by rifampin. Rifampin treatment increased intestinal P-gp content 3.5 ± 2.1 -fold, which correlated with the AUC after oral digoxin but not after intravenous digoxin. P-gp is a determinant of the disposition of digoxin. Concomitant administration of rifampin reduced digoxin plasma concentrations substantially after oral administration but to a lesser extent after intravenous administration. The rifampin-digoxin interaction appears to occur largely at the level of the intestine. Therefore, induction of intestinal P-gp could explain this new type of drug-drug interaction.

J. Clin. Invest. 104:147-153 (1999).

Introduction

Marked interindividual variability in plasma concentrations of drugs after administration of a fixed dose is often related to differences in drug metabolism. Inhibition and induction of hepatic drug metabolism, and also of prehepatic biotransformation in the intestine, are important and well-established mechanisms for drug interactions. A number of clinically important drug interactions with rifampin have been reported that are caused by its powerful induction of intestinal cytochrome P4503A4 (1, 2). We have observed a 53-year-old patient taking chronic digoxin in whom concentrations of digoxin decreased below detectable limits when he was treated for bacterial endocarditis with rifampin. A literature search identified 2 further case reports of digoxin-rifampin interactions (3, 4), both describing a 50% reduced concentration of digoxin. Induction of digoxin's metabolism as a mechanism underlying this interaction is rather unlikely, because digoxin is eliminated from the body by renal and biliary excretion of unchanged drug and its metabolism is negligible (5, 6). The data could be reconciled with the clinical observation if rifampin, in addition to its effect on drug metabolism, induces expression of the ATP-binding cassette (ABC) transporter P-glycoprotein (P-gp), the *mdr-1* gene product, in the intestine.

Indeed, digoxin has been identified in vitro and animal experiments as a substrate of renal (7, 8) and intestinal P-gp (9, 10). A potential role for P-gp in the intestinal absorption and prehepatic elimination of numerous drugs (e.g., digoxin) has been recognized (11). These experiments suggest a major role for P-gp in the interaction of digoxin with other drugs, such as quinidine (12, 13). After administration of digoxin to *mdr1a*^{-/-} mice, concentrations of digoxin in brain and plasma were 35- and 2-fold higher, respectively, compared with wild-type (10). Other data from P-gp knockout mice indicate a considerable reduction of direct intestinal secretion of digoxin into the gut lumen in knockout mice but no significant decrease in biliary and renal excretion of digoxin (14). Both its location at the apical membrane of enterocytes and its function as an efflux pump suggest a particular role of intestinal P-gp for the disposition of digoxin. Inhibition of intestinal P-gp by oral treatment with the P-gp inhibitor PSC833 (18) increases bioavailability of concomitantly administered drugs. In contrast to such inhibition, recent experimental evidence obtained from human colon carcinoma cell lines indicates that P-gp expression is upregulated by rifampin (19). If rifampin induces intestinal P-gp in humans, and digoxin is transported from apical duodenal cells during absorption back into the lumen of the intestine, then coadministration of

rifampin should affect digoxin disposition predominantly after oral administration. Taken together, animal experiments and limited clinical data suggest the possibility of a clinically important interaction of digoxin and rifampin based on induction of intestinal P-gp.

To test this hypothesis, we investigated the pharmacokinetics of digoxin in 8 healthy male volunteers before and during concomitant rifampin therapy. Each volunteer underwent endoscopy before and after rifampin therapy to obtain small-bowel biopsy specimens for determination of individual P-gp expression in the enterocytes. We present experimental evidence that intestinal expression of P-gp affects plasma concentrations of orally administered digoxin and that rifampin decreases plasma concentrations of digoxin by induction of intestinal P-gp.

Methods

Human subjects. Eight healthy male volunteers (age 29 ± 5 years; body weight 84 ± 9 kg [mean \pm SD]) were included in the study. All subjects gave their written, informed consent. The study protocol had been approved by the local ethics committee (Stuttgart, Germany). No subject took medications, and all refrained from consumption of caffeine and alcohol for the duration of the study.

Protocol. After an overnight fast at the beginning of the study, all 8 volunteers underwent esophagogastroduodenoscopy (EGD) without any sedation. Biopsies of small-bowel mucosa (second portion of the duodenum) were obtained and immediately placed in forma-

lin for immunohistochemistry, or were directly snap-frozen in liquid nitrogen for Western blot analysis. On the day after EGD (day 2), volunteers were randomized to a single oral dose of 1 mg digoxin ($n = 4$) or an intravenous infusion of 1 mg digoxin ($n = 4$; Lanicor; Boehringer Mannheim GmbH, Mannheim, Germany) over 30 minutes. The volunteers abstained from food for 10 hours before and 4 hours after digoxin administration. Venous blood samples (8 mL) were collected before and 0.17, 0.33, 0.5, 0.58, 0.67, 0.75, 1, 1.25, 1.5, 2, 3, 5, 7, 9, 12, 24, 48, 72, 96, 120, and 144 hours after administration of digoxin. Blood samples were centrifuged, and separated plasma was stored at -20°C . Total urine was collected for 7 days. An electrocardiogram and the automated blood pressure were recorded continuously. On day 8, the volunteers took 600 mg rifampin (RIFA; Grünenthal GmbH, Stolberg, Germany) once daily orally until day 23. On day 17, all 8 volunteers underwent a second EGD. The next day (day 18), they received the oral or intravenous dose of digoxin in the same manner as on day 2, with identical blood and urine sampling. After a washout period of 12 weeks, the identical protocol was repeated, except for a switch of digoxin administration (volunteers who received the oral dose first were now treated intravenously, and vice versa).

Assay of digoxin. Plasma and urine concentrations were determined by an automatic fluorescence polarization immunoassay (TDx/TdxFLx; Abbott Laboratories, North Chicago, Illinois, USA). The limit of quantification was 0.1 ng/mL. All plasma and urine samples from each patient were assayed together with calibration and quality control. The inter- and intraday coefficient of variation at plasma levels of 1.5 ng/mL was $<4\%$.

Immunohistochemical staining. From each duodenal specimen, 2.5- μm -thick paraffin sections were prepared by standard methods. A rabbit polyclonal antibody raised against human CYP3A4 (20) recognizing the CYP3A subfamily was used for immunostaining that was performed using a modified ABC technique (21, 22). The primary antibody and secondary biotinylated anti-rabbit antibody were both diluted 1:200. Neuraminidase (acetate buffer [pH 5.4], 37°C ; Behring, Marburg, Germany), diluted 1:50, was used for 120 minutes as enzyme pretreatment. Viewing of the ABC complex was achieved with H_2O_2 and diaminobenzidine. All samples were immunostained in the same batches under identical conditions, to reduce intraday variability of the immunostaining method. For detection of villin, an mAb (1:100 dilution; Chemicon International, Temecula, California, USA) was used. Otherwise, immunostaining was performed as already described here. For detection of P-gp, we used the monoclonal anti-P-gp antibody F4 (1:200 dilution; Sigma Chemical Co., St. Louis, Missouri, USA) (23). Similar staining results were obtained with another antibody raised against P-gp (C219). Renal tissue samples were used as positive controls for P-gp immunostaining. The secondary antibody was rabbit anti-mouse, diluted 1:100. For determining P-gp, we used the alkaline phosphatase/anti-alkaline phosphatase (APAAP) method (24).

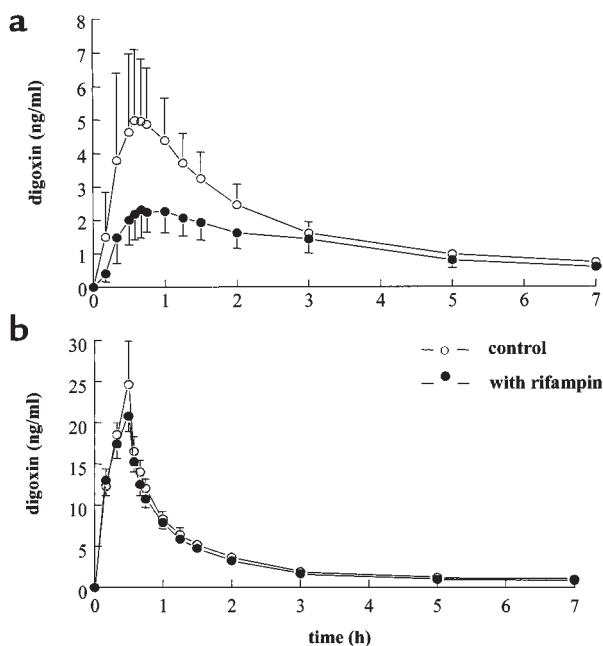


Figure 1
(a) Mean ($n = 8$) plasma concentration (mean \pm SD) time curves of orally administered digoxin (1 mg) before (open circles) and during (filled circles) coadministration of rifampin (600 mg). **(b)** Mean ($n = 8$) plasma concentration (mean \pm SD) time curves of intravenously administered digoxin (1 mg) before (open circles) and during (filled circles) coadministration of rifampin (600 mg).

Quantitative assessment of immunohistochemistry. For quantification, we used an image analysis workstation (Histoanalyzer) as described previously (22, 25). Briefly, the instrumental design of the Histoanalyzer equipment consists of a 3CCD color video camera (Sony Corp., Tokyo, Japan), a Type 020-452.008 microscope (Leitz Aristoplan, Wetzlar, Germany) with a scanning table (Merzhäuser, Wetzlar, Germany) and a workstation (Sun Microsystems, Palo Alto, California, USA). Measurements were performed with a $\times 40$ objective. OD was measured in the blue channel of the red/green/blue camera signal. The field of interest, the luminal membrane of the enterocytes (see Figure 3), was labeled with a cursor mouse system, with the results read as OD/ μm^2 . The following controls were performed for the quantitative immunohistochemical measurements: coefficient of variation of the optical system and the sample. The former was assessed by a 10-fold measurement of the mean OD in 1 area of interest (0.2%), and the second by a 10-fold measurement of mean OD in different fields of interest in 1 duodenal biopsy (0.9%). The analysis was carried out in a blinded fashion by an independent investigator.

Western blot analysis of P-gp. Biopsy specimens (~5 mg wet weight each) were homogenized in 200 μL homogenizing buffer (10 mM Tris [pH 7.4], 90 mM NaCl, 1 mM NaEDTA, 1 mM Pefa-Bloc, 1 $\mu\text{g}/\text{mL}$ pepstatin) using a 2-mL conical Wheaton glass tube with a motor-driven Teflon pestle (1,000 rpm for 2 minutes). Protein concentrations were determined according to the method of Smith et al. (26). A total of 50 μg of whole-biopsy homogenate were electrophoresed on a 12% SDS-PAGE gel without heating the samples. L-MDR1 (2 and 10 μg) and LLC-PK1 (25 μg) (10) cell homogenates (both provided by Alfred Schinkel, The Netherlands Cancer Institute, Amsterdam, the Netherlands) were also loaded as positive and negative controls. Transfer of proteins to PVDF membrane (Roche Diagnostics, Mannheim, Germany) was carried out in a tank blotter (Trans Blot Cell; Bio-Rad Laboratories Inc., Hercules, California, USA). The blot was then rinsed once with Tris-buffered saline (TBS) and stored in blocking buffer-1 (TBS with 5% nonfat dry milk and 0.1% Tween-20) overnight. The next morning, the blot was incubated with a monoclonal anti-P-gp antibody F4 (Sigma Chemical Co.) (23) for 1 hour. The blot was washed 3 times with washing buffer (TBS with 0.1% Tween-20) and then incubated with peroxidase-labeled (POD-labeled) anti-mouse IgG (100 mU/mL in blocking buffer-1; Roche Diagnostics). After washing the blot 3 times with washing buffer, it was then developed with an enhanced chemiluminescence (ECL) kit and exposed to ECL Hyperfilms (Amersham Pharmacia Biotech, Freiburg, Germany) for 30 seconds. Antibodies were stripped off the blot by incubating for 30 minutes at 50 °C in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62 mM Tris-HCl [pH 6.7]). After blocking the blot in blocking buffer-2 (1% purified

Table 1

Pharmacokinetic parameters after intravenous and oral administration of 1 mg digoxin before and during concomitant treatment with rifampin (600 mg)

	Digoxin 1 mg oral		Digoxin 1 mg intravenous	
	Control	With rifampin	Control	With rifampin
AUC _(0-3 h) (ng/h/mL)	8.8 ± 2.9	5.0 ± 1.1 ^A	22.1 ± 1.7	20.0 ± 2.2
AUC _(0-144h) (ng/h/mL)	54.8 ± 11.6	38.2 ± 12.4 ^B	87.3 ± 8.3	74.5 ± 10.5 ^B
F (%)	63 ± 11	44 ± 14 ^B		
T _{max} (min)	42 ± 12	52 ± 18 ^B		
C _{max} (ng/mL)	5.4 ± 1.9	2.6 ± 0.7 ^A	24.7 ± 5.2	20.9 ± 1.8
CL _R (mL/min)	159 ± 30	159 ± 38	151 ± 25	147 ± 18
CL _{NR} (mL/min)			17 ± 17	54 ± 29 ^A
Half-life (h)	56 ± 13	54 ± 13	58 ± 12	53 ± 11

AUC, area under the plasma concentration time curve up to 3 or 144 hours; C_{max}, maximum plasma concentration; CL_R, renal clearance; CL_{NR}, nonrenal clearance; F, bioavailability; T_{max}, time of maximum plasma concentration. ^AP < 0.01. ^BP < 0.05.

casein [Roche Diagnostics] in TBS) for 1 hour, CYP3A detection was carried out using a rabbit polyclonal 3A antibody (20) in TBS containing 0.1% Tween-20 and POD-labeled anti-rabbit IgG (Roche Diagnostics) in 100 mU/mL in blocking buffer-2, as already described here.

Developed films were scanned with a ELScript400 densitometer (Hirschmann Instruments, Neuried, Germany), and ODs were calculated using ZeroDScan software (Scanalytics, Billerica, Massachusetts, USA).

Pharmacokinetic calculation. The area under the plasma concentration time curve (AUC) of digoxin was calculated, by use of the trapezoidal rule, from 0 to 3 and 0 to 144 hours after administration. Systemic clearance (CL) was calculated from dose per AUC. Peak digoxin concentrations (C_{max}) and the times when they occurred (T_{max}) were derived directly from the data. Elimination half-life (t_{1/2}) was estimated based on the terminal log-linear data points. The renal clearance (CL_R) was calculated by $CL_R = Ae_{0-144h}/AUC_{0-144h}$, where Ae_{0-144h} represents amount of digoxin excreted in urine. The nonrenal clearance (CL_{NR}) was calculated by $CL_{NR} = CL - CL_R$.

Creatinine clearance. For each volunteer in each study arm before and during rifampin treatment, creatinine clearance was determined by standard methods using the ratio of the creatinine concentration in urine over creatinine plasma concentration multiplied by the 24-hour urine volume.

Statistical analysis. Quantitative immunohistochemistry and pharmacokinetic parameters for digoxin (without and with coadministration of rifampin) were compared with Mann-Whitney tests, using GraphPad InStat software (GraphPad Software for Science Inc., San Diego, California, USA). Changes in T_{max} and P-gp levels were tested with the nonparametric Wilcoxon rank test. The correlation of duodenal P-gp and plasma AUC of digoxin was tested with the nonparametric Spearman rank test. All data are presented as mean ± SD.

Results

Pharmacokinetics. After administration of rifampin, AUC of oral digoxin was significantly decreased (P < 0.05). Maximal plasma levels were reduced by 58% (P < 0.01), and T_{max} increased from 42 to 52 minutes

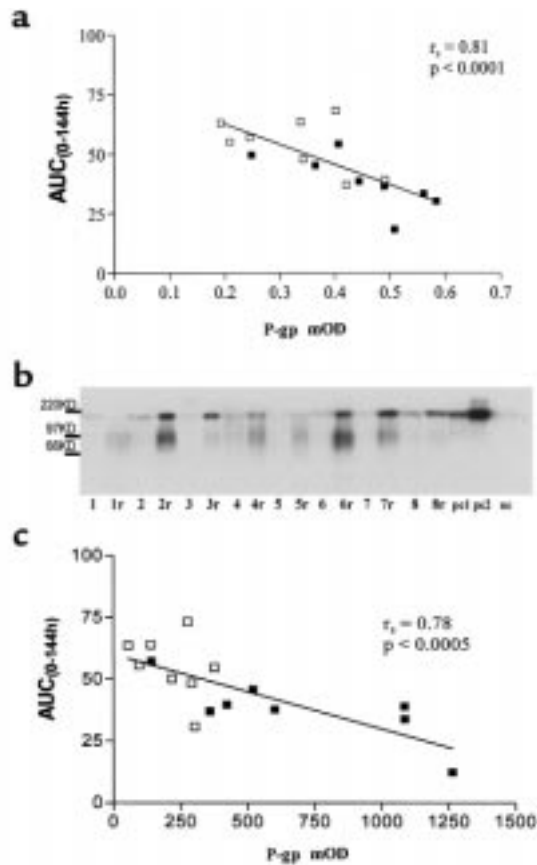


Figure 2
 (a) Correlation between AUC of orally administered digoxin (1 mg) and expression of P-gp ($n = 16$), measured by immunohistochemistry using TV-densitometry before and after coadministration of rifampin. Open squares = without rifampin; closed squares = with rifampin (600 mg). (b) Western blot of biopsy homogenates showing the induction of P-gp expression. Lanes 1–8, 50 μ g biopsy homogenate of volunteers 1–8 taken before rifampin treatment; lanes 1r–8r, 50 μ g biopsy homogenate of volunteers 1–8 taken after rifampin treatment; lanes pc1 and pc2, L-MDR1 cell homogenates (positive control, 2/10 μ g); nc, LLC-PK1 cell homogenate (negative control, 25 mg). (c) Correlation between AUC of orally administered digoxin (1 mg) and expression of P-gp ($n = 16$) measured by Western blot. Open squares = without rifampin; filled squares = with rifampin (600 mg).

($P < 0.05$; Figure 1a and Table 1). Oral bioavailability (F) of digoxin decreased by 30.1% during rifampin therapy ($P < 0.05$). A smaller, although still significant, change ($P < 0.05$) was noted for the decrease of plasma AUC after intravenous administration, with digoxin's systemic clearance increasing by 21% ($P < 0.05$) (Figure 1b and Table 1). Renal clearance of digoxin and creatinine clearance were not altered by rifampin. Cumulative urinary excretion of orally and intravenously administered digoxin was lowered by 31% and 17%, respectively, during rifampin coadministration. No significant difference was detected in the terminal half-life.

P-gp, CYP3A, and villin expression in the duodenum. Expression of P-gp and CYP3A in enterocytes as assessed by quantitative immunohistochemistry was significantly higher during rifampin therapy ($P < 0.05$

and $P < 0.001$, respectively; Table 2). Expression of villin was not altered by rifampin coadministration. The expression of CYP3A did not correlate with interindividual differences in pharmacokinetic data of digoxin. In contrast, differences in P-gp expression in enterocytes of duodenal biopsies correlated significantly with plasma AUC of oral digoxin, both for the entire set of data ($r_s = -0.81$, $P < 0.0001$, $n = 16$; Figure 2a) and the interindividual differences in the rifampin treatment group ($r = -0.81$, $P < 0.05$, $n = 8$), whereas no correlation was found in the treatment group without coadministration of rifampin reflecting basal P-gp expression.

Western blot. Western blot analysis of P-gp confirmed the results obtained by immunohistochemistry. In fact, comparison of P-gp levels determined by independent methods of immunohistochemistry and Western blot revealed a highly significant correlation ($r_s = 0.78$, $P < 0.0005$).

Intestinal P-gp levels were induced 3.5 ± 2.1 -fold by rifampin (Table 3). Consistent with data from immunohistochemistry, intestinal P-gp expression determined by Western blot and plasma AUC of oral digoxin correlated significantly in the rifampin treatment group ($r_s = -0.78$, $P < 0.05$), as well as in the entire set of data ($r_s = -0.78$, $P < 0.0005$; Figure 2b).

Intestinal CYP3A content was found to be increased 4.4 ± 2.7 -fold (Table 3). A borderline correlation between plasma AUC of oral digoxin and intestinal CYP3A levels ($r_s = -0.54$, $P < 0.05$) was observed when the data were analyzed as a whole, but no such correlation existed when the 2 study groups were analyzed separately.

Discussion

The present study describes a substantial drug-drug interaction between rifampin and digoxin. Concomitant administration of the antibiotic has a profound effect on the rate and extent of absorption of digoxin administered orally, whereas these effects are less pronounced after intravenous administration.

Several mechanisms could contribute to this interaction. First, rifampin is a known inducer of cytochrome P450 enzymes (e.g., CYP3A4 [27]), and induction could result in a substantial fraction of digoxin being metabolized via these enzymes. Several lines of evidence, however, argue against a major contribution of metabolism to the digoxin-rifampin interaction. First, induction of hepatic metabolism of digoxin would decrease half-life both after systemic and oral administration, an effect that has not been observed in the present study. Prehepatic metabolism

Table 2

Expression of villin, CYP3A, and P-gp in enterocytes of duodenal biopsies before ($n = 8$) and after ($n = 8$) administration of rifampin, as determined by quantitative immunohistochemistry

	Control Mean OD	With rifampin Mean OD	Ratio Rifampin/control	P value
Villin	0.28 \pm 0.41	0.22 \pm 0.23	1.3 \pm 0.2	Not significant
CYP3A	1.21 \pm 0.33	2.33 \pm 0.12	2.2 \pm 0.2	$P < 0.001$
P-gp	0.34 \pm 0.10	0.44 \pm 0.10	1.4 \pm 0.5	$P < 0.05$

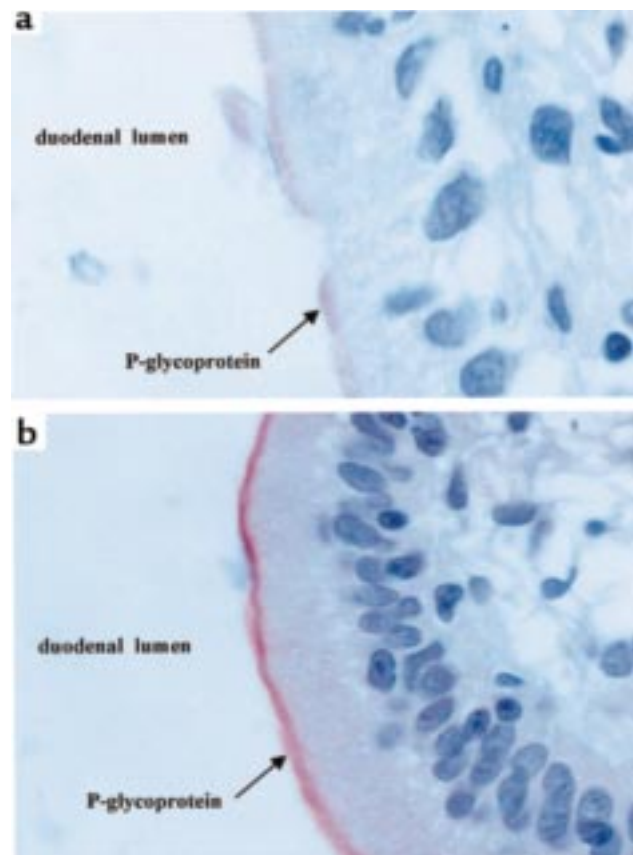
Table 3

Expression of CYP3A and P-gp in enterocytes of duodenal biopsies before ($n = 8$) and after ($n = 8$) administration of rifampin, as determined by Western blot

	Control	With rifampin	Ratio	
	OD	OD	Rifampin/control	<i>P</i> value
CYP3A	118 ± 88.1	296 ± 269	4.4 ± 2.7	$P < 0.005$
P-gp	273 ± 160	499 ± 467	3.5 ± 2.1	$P < 0.005$

(i.e., biotransformation of digoxin in the gut wall) could also increase apparent oral clearance, and would be consistent with the less pronounced interaction after intravenous administration. This phenomenon has been described by our group for the calcium channel blocker verapamil, a drug that undergoes extensive prehepatic metabolism via CYP3A4. However, such prehepatic metabolism for digoxin, a low-clearance compound, has not been described. In the present study, we quantified duodenal CYP3A expression. CYP3A in duodenal samples was enhanced 4.4-fold after rifampin induction, similar to recent reports from other groups (27). The individual expression of CYP3A determined with 2 independent methods in samples obtained from biopsies, however, only revealed a borderline correlation ($r_s = -0.54$, $P < 0.05$, $n = 16$) with interindividual AUC and, hence, with clearance of digoxin, when determined by Western blot. No such correlation was found with CYP3A determined by immunohistochemistry. Given that in vitro metabolism of digoxin in human primary hepatocytes and liver microsomes has been shown not to be cytochrome P450 dependent (6), induction of metabolism cannot be held responsible for the observed changes in digoxin's extent and rate of absorption. In contrast, quantitative immunohistochemistry and Western blotting of intestinal P-gp were strong predictors of digoxin clearance ($r_s = -0.81$, $P < 0.0005$), both for the entire population and for the interindividual differences in the rifampin treatment group ($r = -0.81$, $P < 0.05$) (Figure 2a). These findings were confirmed by Western blot analysis of P-gp (Figure 2b). We observed a 3.5-fold increase in intestinal P-gp expression after coadministration of rifampin. In an in vitro experiment using human colon carcinoma cells, it was also demonstrated that rifampin can induce the expression of P-gp (19). These data strongly indicate that P-gp modulates digoxin disposition, leading to low drug concentrations in individuals with high P-gp expression and high concentrations in those with low P-gp expression. Accordingly, rifampin-mediated P-gp induction is associated with a reduction in plasma digoxin. The pharmacokinetic consequences of this interaction are readily explained by a P-gp-based mechanism. After absorption into the enterocyte, digoxin is pumped back into the lumen via P-gp, and, therefore, the major effect has to be expected during the first hours after administration. In fact, digoxin AUC after oral administration was mainly reduced during the first 3 hours, whereas plasma concentra-

tions from 3 to 144 hours are almost superimposable. We observed a shift in T_{max} of digoxin after rifampin coadministration that can also be attributed to increased P-gp expression and is not observed under conditions of increased presystemic metabolism. Based on this hypothesis, digoxin disposition after intravenous administration should be less affected by rifampin, as only a minor fraction of the dose will reach the enterocytes. As displayed in Figure 1b and Table 1, plasma concentrations of intravenous digoxin are reduced by only 15% after coadministration of rifampin. Direct secretion of compounds such as digoxin through the gut wall by P-gp has been identified as an important pathway of drug elimination by several groups (10, 14, 28). For example, Mayer and coworkers observed that ~16% of intravenously administered digoxin was directly excreted into the intestinal lumen within 90 minutes in wild-type mice, compared with ~2% in *mdr1a*^{-/-} mice (14). Our study indicates this pathway to be of minor importance in humans, as we find limited effects of rifampin after intravenous administration of digoxin, and no correlation between intestinal P-gp expression and digoxin's systemic clearance. Mayer and coworkers also reported that biliary and renal excretion of digoxin was

**Figure 3**

(a) Duodenal biopsy (villus tip, ×40) immunostained for P-gp before administration of rifampin. (b) Duodenal biopsy (villus tip, ×40) immunostained for P-gp after 9 days administration of rifampin (600 mg), obtained from the same volunteer as in a.

not reduced to a similar extent in P-gp knockout animals, indicating that other mechanisms contribute to digoxin's elimination in these organs. These animal data are in good agreement with our findings in humans, indicating an important role for intestinal P-gp in digoxin disposition, whereas a possible induction of P-gp in liver and kidney did not influence pharmacokinetics of digoxin in our investigation. Taken together, pharmacokinetics, quantitative immunohistochemistry, and Western blots strongly suggest that intestinal P-gp plays a key role in systemic availability of digoxin, as was demonstrated for the interpatient variation in the oral bioavailability of cyclosporin (29).

The present study addresses rifampin-modulated P-gp expression in the duodenum and its consequences for drug disposition. Given that P-gp is constitutively expressed in other organs or organ systems, the question arises whether rifampin treatment modulates nonintestinal P-gp. Several recent studies describe the inhibition of renal P-gp by coadministration of drugs, resulting in higher plasma concentrations of digoxin (30–32). These studies indicate that P-gp plays a major role in renal and intestinal excretion (33) and secretion (9) of digoxin, and that the inhibition can explain the yet unknown mechanism of clinically relevant interactions (e.g., digoxin-quinidine [32], digoxin-verapamil [34], digoxin-cyclosporin A [31], digoxin-propafenone [35], and digoxin-itraconazole [36]). Mechanistically, such interactions are explained by competitive inhibition of P-gp, resulting in reduced net transport into the proximal tubules of the kidney. Therefore, a potential induction of renal P-gp by rifampin should increase renal clearance of digoxin. In contrast to this assumption, we observed no change of digoxin renal clearance during rifampin therapy, thereby indicating the possibility of tissue-specific regulation of P-gp by rifampin. A possible explanation for the lack of P-gp upregulation in the liver and kidney could be the higher rifampin levels to which only enterocytes are exposed after oral administration. As already discussed here, the involvement of other excretion mechanisms in the kidney and liver could alternatively mask an induction of P-gp in these organs. Recently, it was reported that rifampin is a ligand and activator of the human glucocorticoid receptor (37), which may in part explain its effects on P-gp expression. Therapy with glucocorticoids has been shown to increase P-gp in nasal mucosa, blood-brain barrier, hepatoma cells, and lymphocytes during glucocorticoid therapy (38, 39). The question of rifampin-mediated upregulation of P-gp in other organs (e.g., in the blood-brain barrier), which could have important consequences for central nervous system side effects of digoxin, requires further studies.

In conclusion, the results of the present study support the idea that P-gp in the epithelium of the gut wall determines plasma concentration of orally administered digoxin and that rifampin induces intestinal P-gp expression in humans. This interaction is of clinical relevance given the renewed popularity of rifampin and the numerous interactions arising from rifampin-mediated P-gp induction.

Acknowledgments

We thank Alfred Schinkel for providing us with L-MDR1 and LLC-PK1 cells. This work was supported by grants from the Robert Bosch Foundation and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF grant 01EC9405).

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