Complementary Stimulation of Hepatobiliary Transport and Detoxification Systems by Rifampicin and Ursodeoxycholic Acid in Humans

HANNS-ULRICH MARSHALL,* MARTIN WAGNER, ‡ GERNOT ZOLLNER, ‡ PETER FICKERT, ‡ ULF DICZFALUSY, § JUDITH GUMHOLD, ‡ DAGMAR SILBERT, ‡ ANDREA FUCHSBICHLER, ‡ LISBET BENTHIN,* ROSITA GRUNDSTRÖM, ¶ ULF GUSTAFSSON, ¶ STAFFAN SAHLIN, ¶ CURT EINARSSON,* and MICHAEL TRAUNER ‡

*Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden; ‡Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Medicine, Medical University, Graz, Austria; §Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden; ¶Institute of Pathology, Medical University, Graz, Austria; and ¶Department of Surgery, Danderyds Hospital, Stockholm, Sweden

Background & Aims: Rifampicin (RIFA) and ursodeoxycholic acid (UDCA) improve symptoms and biochemical markers of liver injury in cholestatic liver diseases by largely unknown mechanisms. We aimed to study the molecular mechanisms of action of these drugs in humans.

Methods: Thirty otherwise healthy gallstone patients scheduled for cholecystectomy were randomized to RIFA (600 mg/day for 1 week) or UDCA (1 g/day for 3 weeks) or no medication before surgery. Routine biochemistry, lipids, and surrogate markers for P450 activity (4\(\beta\)-hydroxy cholesterol, 4\(\beta\)-OH-C) and bile acid synthesis (7\(\alpha\)-hydroxy-4-cholesten-3-one, C-4) were measured in serum. Bile acids were analyzed in serum, urine, and bile. A wedge liver biopsy specimen was taken to study expression of hepatobiliary ABC transporters as well as detoxification enzymes and regulatory transcription factors.

Results: RIFA enhanced bile acid detoxification as well as bilirubin conjugation and excretion as reflected by enhanced expression of CYP3A4, UGT1A1, and MRP2. These molecular effects were paralleled by decreased bilirubin and deoxycholic acid concentrations in serum and decreased lithocholic and deoxycholic acid concentrations in bile. UDCA on the other hand stimulated the expression of BSEP, MDR3, and MRP4. UDCA became the predominant bile acid after UDCA treatment and lowered the biliary cholesterol saturation index.

Conclusions: Rifampicin (RIFA) enhances bile acid detoxification as well as bilirubin conjugation and export systems, whereas UDCA stimulates the expression of transporters for canalicular and basolateral bile acid export as well as the canalicular phospholipid flippase. These independent but complementary effects may justify a combination of both agents for the treatment of cholestatic liver diseases.

Ursodeoxycholic acid (UDCA) improves clinical and biochemical serum parameters in a variety of cholestatic liver diseases.¹ UDCA is nowadays considered the first-line treatment for patients with chronic cholestatic liver diseases such as primary biliary cirrhosis (PBC) because a combined analysis of the 3 largest randomized clinical trials of UDCA in PBC indicates that UDCA improves survival free of liver transplantation.² However, the efficacy of UDCA treatment has been debated,³,⁴ and the mechanism(s) of action in humans are still not defined.¹ Suggested mechanisms of UDCA include improved bile acid transport and/or detoxification, cytoprotection, and antiapoptotic effects.¹

Before the era of UDCA, there have been small trials with rifampicin (RIFA) in cholestatic liver disease, which all showed a marked improvement of pruritus.⁵⁻¹¹ In PBC, RIFA also significantly decreased serum levels of transaminases,⁷ \(\gamma\)-glutamyltransferase,⁷,¹⁰ alkaline phosphatase,⁷ and total bile salts.⁵⁻⁷ Nowadays, RIFA is mainly used for treatment of pruritus not responding to bile acid sequestrates.¹² A proposed molecular mecha-

Abbreviations used in this paper: 4\(\beta\)-OH-C, 4\(\beta\)-hydroxy cholesterol; BAAT, bile acid amino transferase; BSEP, bile salt export pump; C4, 7\(\alpha\)-hydroxy-4-cholesten-3-one; CA, cholic acid; CYP, cytochrome P450; DCA, deoxycholic acid; HCA, hyocholic acid; LCA, lithocholic acid; CDCA, chenodeoxycholic acid; HDCA, hyodeoxycholic acid; MDR, multidrug resistance protein; MRP, multidrug resistance-related protein; OATP, organic anion transporting polypeptide; PBC, primary biliary cirrhosis; RIFA, rifampicin; UGT, uridine diphosphate glucuronosyltransferase; UDCA, ursodeoxycholic acid.

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nism of action of RIFA is the hydroxylation of hydrophobic bile acids, thereby decreasing their cytotoxicity, an effect mediated by stimulation of CYP3A expression via activation of the nuclear receptor SXR/PXR in mice.\textsuperscript{13,14} Moreover, decreased expression of the key enzyme of bile acid synthesis, \textit{Cyp7a1}, was observed in rodents.\textsuperscript{15} Again, the anticholestatic and antipruritic mode of action of RIFA in humans remains to be determined.

To identify potential molecular mechanism(s) of action of UDCA and RIFA in humans, we performed a comprehensive biochemical and molecular study of the effects of these agents on bile acids, biliary lipids, and genes involved in bile acid/bilirubin metabolism\textsuperscript{15} and transport\textsuperscript{16} in otherwise healthy patients with gallstone disease.

**Patients and Methods**

**Patients**

Otherwise healthy patients scheduled for laparoscopic cholecystectomy for symptomatic gallstone disease were invited to participate in a clinical study of the metabolic and molecular effects of UDCA and RIFA. This approach was taken because such a detailed analysis of liver, bile, serum, and urine is not feasible in patients not scheduled for a surgical abdominal procedure. Potential candidates completed a clinical research file consisting of a detailed questionnaire about the patient’s history and lifestyle. Previous or ongoing liver, kidney, intestinal, or metabolic diseases were exclusion criteria as well as the use of medications known to affect liver function and metabolism, eg, lipid or glucose lowering drugs, P450 enzyme inducers.

After giving a written informed consent, 30 patients between 47 and 58 years of age were randomized to RIFA (Rimactan, Sandoz, Basel, Switzerland, 600 mg/day for 1 week) or UDCA (Ursofalk, Dr Falk, Freiburg, Germany, 1 g/day for 3 weeks) or no medication (controls) before surgery. Study drugs were administered open-labeled in 1 (RIFA) or 2 (UDCA) daily doses until the day before surgery. The study protocol was approved by the ethics committee at Karolinska Institutet. The prevalence of cholesterol or pigment gallstones was judged visually (by C.E.).

**Clinical Chemical and Bile Acid Analyses in Serum and Urine**

Routine parameters including serum lipids and lipoproteins as well as liver and kidney function tests were analyzed using standard clinical biochemical procedures. Serum \(4\beta\)-hydroxycholesterol (\(4\beta\)-OH-C), a marker of CYP3A4,\textsuperscript{17} \(7\alpha\)-hydroxy-\(4\beta\)-cholesten-\(3\)-one (C-\(4\)), a sensitive marker of endogenous bile acid synthesis,\textsuperscript{18} as well as serum bile acids were analyzed by isotope-dilution gas chromatography-mass spectrometry (GCMS) as described previously.\textsuperscript{18–20} From morning urine samples, bile acids were isolated using solid-phase extraction and screened for conjugates using electrospray mass spectrometry (ESMS), which is a highly sensitive method for detection of different types of conjugated bile acids, as previously described.\textsuperscript{21} These analyses were performed at the beginning and at the end of the treatment periods in patients randomized to medication.

**Biliary Lipid Analyses**

During surgery, gallbladder bile was sampled for the determination of cholesterol, phospholipids, and bile acids as described previously.\textsuperscript{22} In addition, bile aliquots were screened for bile acid conjugates other than glycine- or taurine-amidates. For this purpose, bile acids were extracted after disrupting protein adsorption by incubating with 1 mL 0.5 mol/L (NHEt\(_4\))\(_2\)SO\(_4\) at 64°C for 30 minutes and analyzed by ESMS both before and after cleavage of glycine or taurine conjugates with cholyglycine hydrolase.\textsuperscript{21}

**mRNA and Protein Expression Analyses**

A wedge liver biopsy specimen was taken during surgery and immediately frozen in liquid nitrogen. Messenger RNA (mRNA) and protein were then prepared for the determination of mRNA expression levels of enzymes involved in bile acid synthesis and metabolism\textsuperscript{15} (BAAT, CYP3A4, CYP7A1, CYP27, CYP8B1, UGT1A1, UGT2B4, UGT2B7, SULT2A1), regulatory nuclear transcription factors (HNF-\(4\alpha\), PXR/NR1I2, RXR/NR1I1), and mRNA levels (OATP1B1/SLC21A1) and protein as well as mRNA levels of hepatobiliary ABC transporters\textsuperscript{16} (ABCG8, BSEP/ABCB11, MDR3/ABCB4, MRPI/ABCC2, MRP3/ABCC3, MRP4/ABCC4) using recently in-detail-described methodology.\textsuperscript{23–25} Human primer and probe sets used for mRNA estimations by TaqMan real-time PCR and competitive RT-PCR, respectively, are available on request. Liver biopsy specimen material was sufficient to perform the complete set of mRNA expression analyses from all 30 patients included. For Western analyses, sufficient protein was available from all patients serving as controls but only from 9 and 8 patients randomized to RIFA and UDCA, respectively. Protein expression levels from RIFA or UDCA treated patients were estimated in comparison with proteins prepared from controls, with a sample from a patient assigned to RIFA or UDCA treatment groups serving as reference (R in Figures 4 and 5). To verify that antibodies used for Western analyses estimated expression of membrane-targeted ABC transport proteins, tissue distribution of canalicular transporters BSEP, MDR3, and MRPI was investigated by immunofluorescence labeling and confocal laser scanning microscopy as described.\textsuperscript{26}

**Statistical Analysis**

Data are expressed as mean values ± standard deviation (SD). Differences within patient groups were analyzed using Student paired \(t\) test and, among patient groups, un-
paired Student t test using the SigmaStat statistic program (Jandel Scientific, San Rafael, CA). A P value of <.05 was considered significant.

**Results**

**Patients’ Demographics, Routine Biochemistry and Type of Gallstones**

The patients randomized to control, RIFA, or UDCA groups did not differ significantly in mean age (range, 29–77 years of age) or body mass index (range, 24.4–35.9 kg/m²). Females outnumbered males (in line with the target population in cholesteric liver diseases such as PBC), and there were more women in the UDCA group (n = 9) than in the RIFA (n = 6) or control (n = 7) groups (Table 1). All patients had cholesterol gallstones.

No adverse effects from study medications were reported. Serum lipids and lipoproteins as well as liver and kidney function tests remained in the normal range in all study patients and did not change significantly from the initial value (Table 2). Of interest, serum bilirubin was the only marker that decreased in each patient in the RIFA group. For the entire RIFA group, this change was statistically significant (P < .05).

**Compliance**

Compliance of all patients randomized to RIFA or UDCA was confirmed by ESMS analyses of urine sample extracts taken at the ends of the study periods. Either RIFA (molecular weight, 822 g/mol) or its metabolites were found with quasimolecular ions between m/z 821 and 682 or N-acetylgalactosamine conjugates of UDCA, with or without additional glycine- or taurine-amidation (see below). In addition, increased formation of 4β-OH-C was found in any patient randomized to study medication (see below and Figure 1).

**Effects of RIFA and UDCA on Biliary Lipids and Bile Acids**

Both RIFA and UDCA significantly increased total lipid concentrations in gallbladder bile because of increased bile acid concentrations (Table 3). However, only UDCA patients had a significantly lower biliary cholesterol saturation index as compared with controls because patients randomized to RIFA also had increased cholesterol concentrations in bile. Phospholipid concentrations did not differ between control, RIFA, and UDCA groups (Table 3).

RIFA significantly lowered secondary but increased primary bile acids in bile, as shown in Figure 2B. Lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and cholic acid (CA) accounted for 1.4% ± 0.9%, 32.8% ± 9.1%, 32.5% ± 6.8%, and 33.0% ± 8.0%, respectively, in bile of controls and 0.6% ± 0.2%, 5.8% ± 4.6%, 44.8% ± 7.1%, and 48.3% ± 8.1%, respectively, in patients administered

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Patients Studied</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Age (y)</td>
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<tr>
<td>BMI (kg/m²)</td>
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</table>

**Table 2. Serum Biochemistry of Patients Studied**

<table>
<thead>
<tr>
<th>Biliary Lipids</th>
<th>Control</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (&lt;5.2 mmol/L)</td>
<td>5.8 ± 0.8</td>
<td>5.6 ± 0.9</td>
<td>5.7 ± 0.9</td>
</tr>
<tr>
<td>LDL cholesterol (&lt;3.4 mmol/L)</td>
<td>3.4 ± 0.8</td>
<td>3.3 ± 0.7</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>HDL cholesterol (&gt;1.4 mmol/L)</td>
<td>1.5 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>APO A1 (&gt;1.4 mmol/L)</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>APO B (&gt;0.8 mmol/L)</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Lp(a) (&gt;0.3 mmol/L)</td>
<td>0.2 ± 0.2</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides (&gt;2.0 mmol/L)</td>
<td>1.5 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Bilirubin (&lt;26 μmol/L)</td>
<td>13.0 ± 2.3</td>
<td>12.3 ± 4.9</td>
<td>7.9 ± 1.9a</td>
</tr>
<tr>
<td>ALAT (&lt;36 U/L)</td>
<td>21.6 ± 5.4</td>
<td>22.8 ± 7.2</td>
<td>28.2 ± 5.4</td>
</tr>
<tr>
<td>ASAT (&lt;36 U/L)</td>
<td>25.2 ± 5.4</td>
<td>26.2 ± 5.4</td>
<td>28.8 ± 3.0</td>
</tr>
<tr>
<td>γGT (&lt;48 U/L)</td>
<td>37.2 ± 28.8</td>
<td>49.2 ± 37.8</td>
<td>45.6 ± 21.0</td>
</tr>
<tr>
<td>ALP (&lt;270 U/L)</td>
<td>178.8 ± 43.2</td>
<td>174.0 ± 41.4</td>
<td>184.2 ± 43.2</td>
</tr>
<tr>
<td>Creatinine (45–84 mmol/L)</td>
<td>77.8 ± 6.4</td>
<td>83.7 ± 13.0</td>
<td>427.2 ± 22.2</td>
</tr>
<tr>
<td>Urea (3–10 mmol/L)</td>
<td>4.4 ± 0.6</td>
<td>5.1 ± 7.3</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>Albumin (34–47 mmol/L)</td>
<td>41.1 ± 1.5</td>
<td>42.7 ± 2.2</td>
<td>43.2 ± 1.8</td>
</tr>
<tr>
<td>Total bile acids (&lt;5 μmol/L)</td>
<td>1.1 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.2</td>
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**NOTE.** Normal range in parentheses.

aP < .05, Student paired t test.
RIFA. In UDCA patients, UDCA contributed 47.4% \( /H11006 \) 9.6% of total bile acids compared with 0.8% \( /H11006 \) 0.9% in controls and 0.6% \( /H11006 \) 0.6% in RIFA patients. In UDCA patients, the relative amounts of DCA (12.2% \( /H11006 \) 9.6%), CDCA (17.0% \( /H11006 \) 9.6%), and CA (22.1% \( /H11006 \) 8.0%) were significantly lower as compared with controls, whereas the relative amount of LCA (1.3% \( /H11006 \) 0.7%) was the same as in controls, very similar to the bile acid composition in patients with PBC after 2-year treatment with UDCA.28 However, only RIFA changed concentrations of primary (higher) and secondary (lower) bile acids in gallbladder bile, as shown in Figure 2.

In native bile extracts from control, RIFA, and UDCA groups, ESMS only showed anions indicative of glycine- or taurine-conjugated di- or trihydroxylated bile acids. Minor anions at \( m/z \) 455 and 471 indicating small amounts (<5%) of sulfated mono- and dihydroxylated bile acids were first seen after treatment of bile acid extracts with cholyglycine hydrolysis. Anions indicative of glycosidic bile acid conjugates, ie, glucuronides, glucosides, or N-acetylgalactosaminides were not recorded.

Effects of RIFA and UDCA on Serum and Urine Bile Acids

In contrast to bile, total serum bile acid concentrations in patients administered RIFA did not differ from controls (Table 2). However, as in bile, these noncholestatic patients had significantly lower DCA concentrations (Figure 2), whereas the concentrations of CDCA and CA were the same in all study groups. Patients treated with UDCA had 2-fold higher \( (P < .05) \) total serum bile acids (Table 2) because of the abundance of UDCA (58.4% \( \pm 12.4\% \)). Serum LCA was not evaluated because of levels <.05 \( \mu \text{mol/L} \).

Screening for bile acid metabolites in native urine extracts using ESMS indicated the renal excretion of similar RIFA metabolites as found in bile (see above). In addition, anions at \( m/z \) 567, 583, 624, and 640 representing nonamidated and glycine-amidated di- and trihydroxy bile acid glucuronides emerged.29 In urine from patients administered UDCA, anions at \( m/z \) 594, 651, and 701 representing nonamidated and glycine- or taurine-amidated dihydroxy bile acid \( N\)-acetylgalactosamine conjugates21,24 were recorded (see above). No ions indicative of any bile acid metabolite were recorded in urine of patients before treatment or controls.

Table 3. Biliary Lipid Composition After Treatment With Rifampicin, 600 mg/day in 1 Week, or Ursodeoxycholic Acid, 1 g/day in 3 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RIFA</th>
<th>UDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids (mmol/L)</td>
<td>95.1 ± 26.2</td>
<td>180.2 ± 62.1a</td>
<td>215.6 ± 51.7a</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>9.3 ± 3.5</td>
<td>17.7 ± 7.9a</td>
<td>7.0 ± 2.0</td>
</tr>
<tr>
<td>Phospholipids (mmol/L)</td>
<td>28.0 ± 10.5</td>
<td>31.5 ± 16.8</td>
<td>45.2 ± 16.2</td>
</tr>
<tr>
<td>Bile acids (mmol/L)</td>
<td>58.9 ± 26.9</td>
<td>130.6 ± 47.7a</td>
<td>163.4 ± 37.8a</td>
</tr>
<tr>
<td>Cholesterol Saturation Index (%)</td>
<td>127.1 ± 40.3</td>
<td>172.8 ± 61.7</td>
<td>48.4 ± 5.2a</td>
</tr>
</tbody>
</table>

*P < .05, treatment vs controls, Student paired t-test.

Effects of RIFA and UDCA on Metabolic Genes

RIFA as the prototype cytochrome P450 inducer significantly increased hepatic CYP3A4 gene expression (Figure 3). This was also reflected by the highly significant increase (246.5% \( \pm 65.4\% \) compared with baseline) in the formation of \( 4\beta\)-OH-C (Figure 1). UDCA increased the formation of this metabolite as well (Figure 1) but to a much lesser extent (37.5% \( \pm 13.0\% \)), which was not reflected in increased CYP3A4 gene expression levels (Figure 3). RIFA patients expressed significantly higher CYP3A4 levels in liver compared with baseline, whereas UDCA had a significant increase compared with baseline but not compared with RIFA patients.

Figure 1. Effects of RIFA and UDCA on cytochrome P450 (A) and bile acid synthesis (B). The serum marker of CYP3A4 induction \( 4\beta\)-hydroxycholesterol increased in each patient treated with RIFA or UDCA (A). The serum marker for endogenous bile acid synthesis \( 7a\)-hydroxy-4-cholesten-3-one increased significantly only in the group of patients treated with RIFA (B). *P < .05: significantly different compared with baseline. RIFA, solid line; UDCA, broken line.
lower RXR mRNA levels compared with controls (Figure 3). Genes of HNF-4α and PXR, nuclear factors regulation CYP3A4 expression, and genes of CYP7A1, CYP27, and CYP8B1, enzymes regulating alternative pathways of bile acid synthesis in humans were expressed at similar levels in control, RIFA, and UDCA groups (Figure 3). However, as estimated by serum bile acid precursor C-4, a small but significantly increased bile acid synthesis rate was found in RIFA patients (Figure 1).

Expression levels of genes encoding enzymes for bile acid conjugation with glycine- or taurine (BAAT), sulfate (SULT2A1) or glucuronidate (UGT2B4 and UGT2B7) did not differ between groups. However, expression levels of UGT1A1 coding for bilirubin glucuronosyltransferase were significantly higher in RIFA patients (Figure 3).

**Effects of RIFA and UDCA on Hepatobiliary ABC Transporter Gene and Protein Expression**

RIFA patients expressed significantly higher MRP2 mRNA levels compared with controls (Figure 3) and conversely showed significantly more protein expression (Figure 4) of MRP2, responsible for the excretion of bilirubin glucuronides. Expression levels of other hepatobiliary ABC transporters (ABCG8, BSEP, MDR3, MRP3, MRP4, and OATP1B1) did not differ in RIFA patients from controls.

In UDCA patients, mRNA expression levels of none of the hepatobiliary ABC transporter genes differed from controls. However, in UDCA patients, significantly higher protein levels of dedicated hepatobiliary export proteins were found by Western blotting. These were the canicular BSEP and MDR3 proteins, exporting bile salts and phospholipids, and the basolateral MRP4 protein, exporting bile salt conjugates (Figure 5). Canicular membrane localization of BSEP, MDR3, and MRP2 was confirmed by immunofluorescence microscopy (Figure 6).

**Discussion**

The present study was performed to define the molecular mechanisms of action of 2 drugs—RIFA and UDCA (recently reviewed by Paumgartner and Beuers)—that were shown to improve serum liver biochemistry and symptoms in patients with cholestatic liver diseases such as primary biliary cirrhosis (PBC). We found that RIFA and UDCA both enhance expression of...
distinct but complementary sets of bile acid/bilirubin metabolizing/detoxifying enzymes and their corresponding hepatobiliary ABC transporter proteins.

**RIFA Lowers Serum Bilirubin Levels and Alters Bile Acid Metabolism**

We provide evidence that RIFA improves elimination of bilirubin by coordinately enhancing expression of the bilirubin-conjugating enzyme UGT1A1 and the bilirubin diglucuronide excreting MRP2 protein at the canalicular membrane. In fact, the only biochemical routine parameter that significantly changed during our study was serum bilirubin, a finding that may be explained by these molecular RIFA effects. Our findings in otherwise healthy gallstones patients are in line with data from a long-term study with RIFA in PBC patients in which a trend toward lower bilirubin levels was described and by the observation of a complete remission from cholestasis with very high conjugated hyperbilirubinemia in 3 women on RIFA, 2 of them with benign recurrent intrahepatic cholestasis. However, we herein provide for the first time the potential molecular mechanism underlying this important clinical effect. The enhanced hepatobiliary excretion of conjugated bilirubin via MRP2 may be further assisted by enhanced expression of this protein in luminal membrane of intestinal mucosa.

We also found RIFA to alter significantly bile acid metabolism. Lowering of systemic and presumably intrahepatic levels of bile acids during treatment with RIFA was seen as an explanation for the relief from cholestatic pruritus that was consistently observed in a number of trials with RIFA. However, the underlying molecular mechanisms have so far remained unclear. We found a significant reduction of the secondary bile acid DCA in serum, confirming a recent study by Lütjohann et al. and, in addition, a significant reduction of biliary levels of both hydrophobic secondary bile acids, ie, lithocholic acid (LCA) and DCA. However, the concentrations of primary bile acids, CA and CDCA, and

![Figure 4. Effects of RIFA on hepatic protein expression of hepatobiliary transport proteins. Proteins were isolated from controls, RIFA, or UDCA patients and analyzed by Western blotting as described in the Materials and Methods section. Protein expression levels calculated in relation to indicated (R) reference. P < .05: significantly different compared with controls.](image)

![Figure 5. Effects of UDCA on hepatic protein expression of hepatobiliary transport proteins. Proteins were isolated from controls, RIFA, or UDCA patients and analyzed by Western blotting as described in the Materials and Methods section. Protein expression levels calculated in relation to indicated (R) reference. P < .05: significantly different compared with controls.](image)
in turn also of cholesterol,33 were significantly higher in gallbladder bile. These results are unexpected because 2 recent in vitro studies using the human hepatoblastoma cell line HepG234 and human primary hepatocytes35 showed that RIFA inhibits the key enzyme of bile acid synthesis, CYP7A1 via activation of PXR. However, in our in vivo study, we did not find down-regulation of CYP7A1 gene expression as compared with controls; rather, we found a significantly increased bile acid synthesis as reflected by the precursor C-418 in serum, in accordance with previous observations.32,36 One might also speculate that the higher concentrations of primary bile acids in bile of patients reflect induction by RIFA of another—yet unidentified—canalicular bile salt export system, different from BSEP, the existence of which is suggested by studies in Bsep knockout mice.37

How can the lowered levels of secondary bile acids in gallbladder bile and serum be explained? A possible explanation is the decreased formation of these compounds in the intestine. By acting on the intestinal microflora, RIFA may reduce the formation and return to the liver of LCA,38 but data supporting this mechanism are lacking. An alternative explanation may be the induction of CYP3A4 gene expression via activation of PXR,13,14 which is supported by a recent study with the antiepileptic, nonantibiotic drug carbamazepin.39 CYP3A4 converts LCA and DCA by 6α-hydroxylation to HDCA and HCA. 6α-Hydroxylated bile acids are preferentially 6α-glucuronidated40,41 and finally excreted in urine.42 We have previously shown that administration of RIFA results in an increased excretion of 6α-hydroxylated bile acid glucuronides and a decreased excretion of lithocholic acid sulfate.29 We now provide further support for this mechanism by showing induction of CYP3A4 gene expression in liver and the detection of glucuronides of di- and trihydroxylated bile acids only in the urine but not in bile or serum of patients treated with RIFA. Because of the very high catalytic efficiency toward bile acid 6α-glucuronidation,40 the responsive enzymes UGT2B4 and 7 might not require

**Figure 6.** BSEP, MDR3, and MRP2 immunofluorescence staining in liver biopsy specimens from control, RIFA, and UDCA patients. Immunofluorescence staining was performed with specific primary antibodies against BSEP, MDR3, and MRP2 as described in Zollner et al.26 Canalicular localization of these transporters is maintained under all conditions. Bar = 20 μm.
UDCA Induces Expression of Phase I Metabolic Enzymes and Transporters in Human Liver

UDCA was also found to enhance CYP3A4 activity as reflected by increased serum 4β-OH C levels. However, compared with RIFA, formation of this metabolite by UDCA was minor and not reflected by changes in CYP3A4 gene expression levels or reductions of secondary bile acids. Thus, PXR-dependent induction of CYP3A4 by UDCA as shown in primary human hepatocytes seems to be a minor mechanism of action of UDCA. As to bile acid metabolism, we found enrichment of UDCA by approximately 50% in bile, leading to a significantly lower lithogenic index (48%; in controls, 127%), and by approximately 70% in serum, as well as the abundance of N-acetylglucosamine conjugates as the major metabolites of UDCA in urine. Most important, however, were the effects of UDCA on the expression of hepatobiliary ABC transporter proteins. Because of marked species differences in bile acid composition and transporter regulation between humans and rodents, it was essential to study UDCA effects in humans. We now directly demonstrate for the first time in humans that UDCA enhances protein expression of hepatobiliary membrane transport proteins: the phospholipid transporter MDR3 and the bile salt transporter BSEP at the canalicular site and the alternative bile salt conjugate export protein MRP4 at the basolateral membrane. The changes in MDR3 and BSEP are in accordance with the enhanced excretion of bile acid and phospholipids in patients with primary sclerosing cholangitis during UDCA treatment. From animal studies, it was known that UDCA enhances Bsep and Mrp2 protein expression and insertion into the canalicular membrane. Functionally, Bsep insertion was accompanied by increased excretion of taurocholic acid. Of note, our previous study in mice did not find enhanced gene expression levels of Bsep indicating posttranscriptional regulation of Bsep protein expression, which we now confirm even in humans. In mice, UDCA feeding induced hepatic MRP4 expression. This UDCA effect is also confirmed in our present study in humans. In contrast, UDCA feeding induced Mrp2 and Mrp3 only in mice but not in man as now shown in the present study. The induction of key hepatobiliary transport proteins by UDCA in humans may contribute to its anticholestatic and hepatoprotective effects by stimulating both biliary/canalicular excretory and alternative/basolateral excretory routes for bile acids and other potentially toxic biliary constituents.

Which Conclusions Can Be Drawn From Our Data for Clinical Practice?

UDCA is the first-line treatment of PBC, but its effect on pruritus is notoriously poor. Bile acid seques-
trates are often added under this condition, but these agents may interfere with the resorption of UDCA. Thus, RIFA seems to be more suitable, but clinicians are reluctant to prescribe RIFA because of the risk of drug-induced hepatitis, which has been reported to occur in approximately 10% of patients during long-term treatment. This, of course, warrants careful monitoring of transaminases. Induction of CYP3A4 may also interfere with the metabolism of other prescribed drugs. However, deterioration of cholestasis as suggested from in vitro studies showing RIFA to inhibit the bile salt export pump is unlikely to occur from our data that actually showed higher biliary bile salt concentrations. In fact, elevations of serum bile acids have only been observed after single high-dose RIFA treatment but not in long-
term treatment.\textsuperscript{5,7,8} From our data showing independent and mutual supportive effects on the detoxification (CYP3A4, UGT1A1) and elimination of hepatotoxic compounds (BSEP, MRP2, MRP4) (Figure 7), we speculate that, at least in advanced PBC, a combination therapy with RIFA and UDCA might be more effective than single standard UDCA treatment. Provided the absence of obstructive cholestasis where enhanced biliary excretion of bile acids via BSEP might be deleterious,\textsuperscript{56} combination therapy might be even more important in primary sclerosing cholangitis, which often is complicated by severe pruritus.\textsuperscript{57}

In summary, RIFA was found to enhance elimination of bilirubin by increasing UGT1A1 expression and up-regulation of MRP2 in addition to induce phase I detoxification of bile acids by increased CYP3A4 expression. UDCA is a weak CYP3A4 inducer but significantly enhances expression of hepatobiliary bile salt (BSEP), phospholipid (MDR3), and organic anion-bile salt conjugate/glutathione (MRP4) transporters. These independent but complementary hepatobiliary transporter and enzyme effects support the combined use of both agents for the treatment of advanced cholestatic liver disease.

References


