Protective Effects of 6-Ethyl Chenodeoxycholic Acid, a Farnesoid X Receptor Ligand, in Estrogen-Induced Cholestasis

Stefano Fiorucci, Carlo Clerici, Elisabetta Antonelli, Stefano Orlandi, Bryan Goodwin, Bahman M. Sadeghpour, Giuseppe Sabatino, Giuseppe Russo, Danilo Castellani, Timothy M. Willson, Mark Pruzanski, Roberto Pellicciani, and Antonio Morelli

Clinica di Gastroenterologia ed Epatologia, Dipartimento di Medicina Clinica e Sperimentale Università degli Studi di Perugia, Perugia, Italy (S.F., C.C., E.A., S.O., G.S., G.R. D.C., A.M.); Discovery Research, GlaxoSmithKline, Research Triangle Park, North Carolina (B.G., T.M.W.); Intercept Pharmaceuticals, New York, New York (M.P.); and Dipartimento di Chimica e Tecnologia del Farmaco, Università di Perugia, Perugia, Italy (B.M.S., R.P.)

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ABSTRACT

The farnesoid X receptor (FXR), an endogenous sensor for bile acids, regulates a program of genes involved in bile acid biosynthesis, conjugation, and transport. Cholestatic liver diseases are a group of immunologically and genetically mediated disorders in which accumulation of endogenous bile acids plays a role in the disease progression and symptoms. Here, we describe the effect of 6-ethyl chenodeoxycholic acid (6-ECDCA or INT-747), a semisynthetic bile acid derivative and potent FXR ligand, in a model of cholestasis induced by 5-day administration of 17α-ethynylestradiol (E217α) to rats. The exposure of rat hepatocytes to 1 μM 6-ECDCA caused a 3- to 5-fold induction of small heterodimer partner (Shp) and bile salt export pump (bsep) mRNA and 70 to 80% reduction of cholesterol 7α-hydroxylase (cyp7a1), oxysterol 12β-hydroxylation (cyp8b1), and Na+/taurocholate cotransporting peptide (ntcp). In vivo administration of 6-ECDCA protects against cholestasis induced by E217α. Thus, 6-ECDCA reverted bile flow impairment induced by E217α, reduced secretion of cholic acid and deoxycholic acid, but increased muricholic acid and chenodeoxycholic acid secretion. In vivo administration of 6-ECDCA increased liver expression of Shp, bsep, multidrug resistance-associated protein-2, and multidrug resistance protein-2, whereas it reduced cyp7a1 and cyp8b1 and ntcp mRNA. These changes were reproduced by GW4064, a synthetic FXR ligand. In conclusion, by demonstrating that 6-ECDCA protects against E217α cholestasis, our data support the notion that development of potent FXR ligands might represent a new approach for the treatment of cholestatic disorders.

Cholestasis results in systemic and intrahepatic retention of potentially toxic bile acids that causes liver injury, ultimately leading to biliary fibrosis and cirrhosis (Trauner et al., 1996). Estrogens are well known to cause intrahepatic cholestasis in susceptible women during pregnancy, administration of oral contraceptives, and postmenopausal replacement therapy (Vore, 1987; Bossard et al., 1993; Huang et al., 2000); 2) mdr1a/1b, the two multidrug resistance protein (MDR)-1 isoforms expressed in rodents (Bossard et al., 1993); and 3) the multidrug resistance-associated protein (mrp)-2 (Huang et al., 2000). In addition, estrogen-induced cholestasis associates with a reduced activity of the Na+/taurocholate cotransporting polypeptide (ntcp) (Bossard et al., 1993; Simon et al., 1996) and a reduction of endogenous bile acid synthesis (Simon et al., 1996).
Although physiological actions of estrogens are mediated by the classic signaling pathway in which an estrogen receptor binds to an estrogen response element in the promoter of a gene, recent studies (Lai et al., 2003) have demonstrated that estrogens increase the liver expression of the short heterodimer partner (Shp), an atypical nuclear receptor that lacks a DNA-binding domain and is a target for farnesoid X receptor (FXR) ligands. Shp is known to repress the activity of many nuclear hormone receptors in vitro and is critically involved in regulating bile acid synthesis. Whether Shp activation mediates changes in bile acid synthesis in a rodent model of cholestasis induced by estrogen administration is still unclear.

Bile acids act as signaling molecules that regulate their own biosynthesis and transport by binding to and activating the FXR (Forman et al., 1995; Makishima et al., 1999; Parks et al., 1999; Seol et al., 1995; Wang et al., 1999, 2002). FXR alters the transcription of target genes by binding DNA sequences composed of two inverted repeats separated by one nucleotide as a heterodimer with the 9-cis-retinoic acid receptor α (Forman et al., 1995; Seol et al., 1995). Activation of FXR initiates the transcription of a cohort of genes that function to decrease the concentration of bile acids within the hepatocyte. Specifically, FXR induces the expression of the genes encoding BSEP (Sinal et al., 2000; Ananthanarayanan et al., 2001), MDR3 (Kerr et al., 2002; Wang et al., 2002; Holt et al., 2003), and MRFP. In addition, FXR activation leads to a feedback repression of cyp7a1 and cyp8b1 (Goodwin et al., 2000; del Castillo-Oliva andes and Gill, 2001). These genes encode for the cholesterol 7α-hydroxylase and the sterol 12α-hydroxy-

Materials and Methods

Materials. The synthesis of 6-ECDCA has been described previously (Pelliciari et al., 2002). CDCA and 17α-ethynylestradiol (E217α) were from Sigma-Aldrich (St. Louis, MO).

In Vitro Studies: Effect of 6-ECDCA on Rat FXR-Regulated Genes. Rat hepatocytes were prepared as described previously by in situ collagenase perfusion through the hepatic portal vein (Fiorucci et al., 2002). After isolation, cells were suspended in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 1 mM insulin, 0.15 mg/ml methionine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Cell viability was >86% (trypan blue dye exclusion test). Hepatocytes were then plated in matrix/Matrigel-coated culture plates at a density of 1 × 10^5 cells per dish in 95% air and 5% CO2. Cells were then incubated with 1 μM 6-ECDCA for 24 h, and total RNA was extracted for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis.

In Vivo Studies. Adult male Wistar rats weighing 300 to 350 g were used throughout the study. Before the experiments, the animals were maintained on standard chow and water ad libitum and housed in a temperature (21–23°C) and humidity (45–50%)-controlled room under a 12-h light/dark cycle. All studies were approved by the Animal Study Committee of the University of Perugia.

Surgical Procedures and Bile Flow Measurement. For bile flow measurement, animals were anesthetized with a single dose of sodium pentobarbital (50 mg/kg body wt intraperitoneally) and maintained under this condition throughout the experiment. After catheterization of the jugular vein and carotid artery using a PE-50 polyethylene tubing (Intramedic; Clay Adams, Parsippany, NJ), a middle abdominal incision was made, and the common bile duct was also cannulated (PE-10, Intramedic; Clay Adams). Body temperature was maintained at 37.0 to 38.5°C with a warming lamp to prevent hypothermic alterations of bile flow. Bile flow was determined by gravimetry, assuming a density of the bile of 1.0 g/ml. The biliary excretion rate was calculated as the product between bile flow and biliary concentration. The bile was assayed for both total and individual bile salts by high-pressure liquid chromatography as described previously (Setchell et al., 1997). Total bile acid concentrations were derived by the sum of individual bile acids (Russell and Setchell, 1992).

Estrogen-Induced Cholestasis. Animals were randomly divided into experimental groups (8–12 rats/group) as described in Table 1. For bile collection, surgical procedures were made on the sixth day (i.e., 1 day after the administration of the last dose of E217α). Bile collection started between 9:00 and 11:00 AM to minimize influence of circadian variations. Bile was collected at 15-min intervals for 120 min, and bile flow was determined gravimetrically (Kern et al., 1977; Steiger et al., 1994; Koopen et al., 1999). At the end of the study, the livers were perfused for 30 s with saline, and the major lobe was removed. Liver samples were gently frozen in liquid nitrogen and preserved at −80°C until used for qRT-PCR studies.

Routine Serum Biochemistry and Bile Acid Measurements. Serum samples were stored at −70°C until analysis of aspartate aminotransferase, bilirubin, alkaline phosphatase (ALP), and γ-glutamyl transpeptidase by routine clinical chemistry testing performed on a Hitachi 717 automatic analyzer.

TABLE 1

<table>
<thead>
<tr>
<th>Treatment Regimen</th>
<th>No. of Rats</th>
<th>Duration of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>E17α, 5 mg/kg</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>E17α + 6-ECDCA, 1 mg/kg</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>E17α + 6-ECDCA, 5 mg/kg</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>E17α + 6-ECDCA, 10 mg/kg</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>E17α + CDCA, 10 mg/kg</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>E17α + GW4064, 30 mg/kg</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>
qRT-PCR. After the rats had been killed, livers were removed and immediately snap-frozen on liquid nitrogen and stored at −80°C until use. Total RNA was isolated using TRIzol reagent (Invitrogen, Milan, Italy) as described previously (Fiorucci et al., 2004). Quantification of the expression rat genes was performed by qRT-PCR using the following sense and antisense primers: ntcp, 5'-gcatgagctcttattcaac-3' and 5'-tacatagtgtggccttttggact-3'; bsep, 5'-aagggacttctcttatac-3' and 5'-gtgcactcctcttatac-3'; cyp7a1, 5'-gtgcactcctcttatac-3' and 5'-cacggaaggccatgccagtga-3'; Shp, 5'-cctgggttgctaagggactc-3' and 5'-aatcaacctgatgaagcagagga-3'; FXR, 5'-tgggaatagcagtccgtatcctca; and 5'-atatgatagcagtccgtatcctcaa; Gapdh, 5'-atgccactcctcttatac-3' and 5'-aacactgtatgcaaaccgagga-3'. All PCR primers were designed using software PRIMER3-OUTPUT using published sequence data from the National Center for Biotechnology Information database. The RNA was reverse-transcribed with SuperScript III (Invitrogen) in 20-µl reaction volume using random primers. For RT-PCR, 100 ng of template was used in 25 µL containing 0.3 µM of each primer and 12.5 µL of 2x SYBR Green PCR Master mix (Bio-Rad, Hercules, CA). All reactions were performed in triplicate, and the thermal cycling conditions were as follows: 2 min at 95°C, followed by 50 cycles of 95°C for 10 s and 60°C for 30 s in an iCycler iQ instrument (Bio-Rad). The mean value of the replicates for each sample was calculated and expressed as the cycle threshold (Ct) cycle number at which each PCR reaction reaches a predetermined fluorescence threshold, set within the linear range of all reactions. The amount of gene expression was then calculated as the difference (ΔCt) between the Ct value of the sample for the target gene and the mean Ct value of that sample for the endogenous control (Gapdh). Each gene is expressed as a ratio between control (untreated) and treated rats.

**Statistical Analysis.** Data reported are the mean ± S.E. of the number of experiments indicated. The statistical analysis was carried out using a GraphPad Prism 3 (GraphPad Software Inc., San Diego, CA). Analysis of variance and Student’s t test for paired data were used when appropriate.

**Results**

**6-ECDCA Transactivates FXR and Modulates FXR-Regulated Genes in Hepatocytes.** To examine whether 6-ECDCA has the ability to regulate genes involved in bile acid synthesis and transport, rat hepatocytes were incubated with a maximally effective concentration of 6-ECDCA (1 µM) and its parent molecule CDCA (100 µM) for 24 h, and mRNA expression of Shp, cyp7a1, cyp8b1, bsep, ntcp, and oatp1 was measured by qRT-PCR. As shown Fig. 1B, we found that exposure to FXR ligands increases Shp and bsep mRNA expression by 3- to 5-fold, whereas it reduced cyp7a1, cyp8b1, and ntcp mRNA expression by 50 to 70% (n = 5; P < 0.05 versus control cells). In contrast, 6-ECDCA and CDCA failed to modulate oatp1 gene expression (Fig. 1B). To investigate the effect of estrogen on the gene involved in bile acid synthesis and transport, rat hepatocytes were exposed to 100 nM E217α. As shown in Fig. 1C, exposure to E217α increased Shp mRNA expression by ~2-fold (n = 5; P < 0.05 versus control cells), but it failed to inhibit cyp7a1 and cyp8b1 and ntcp mRNA expression. Thus, in contrast to FXR ligands, induction of liver expression of Shp by E217α does not translate into inhibition of cyp7a1 and cyp8b1.

**6-ECDCA Protects against Cholestasis Induced by E217α.** Five-day administration of E217α slightly decreased body weight (Fig. 2A), which was paralleled by increased levels of serum ALP, a biochemical marker of cholestasis (n = 6–8 animals; P < 0.05 versus control rats) and bile acids. In contrast, E217α treatment failed to modify either ALT, bilirubin, or γ-glutamyl transpeptidase levels (not shown). Administration of 6-ECDCA (Fig. 2, A–D) failed to reverse the body weight loss caused by estrogen but improved serum ALP activity (n = 6–8; P < 0.05 versus E217α at 10 mg/kg/day 6-ECDCA). Treating rats with CDCA did not improve either the body weight, serum ALP or bile acid levels (n = 6–8; P > 0.05 versus E217α). Consistent with these biochemical changes, bile flow analysis carried out 24 h after the last dose of 6-ECDCA or CDCA (Fig. 3, A–C; Table 2) showed a impairment of bile flow that dropped from 68.0 ± 3.4 µl/kg/min in control rats to 36.9 ± 3.5 µl/kg/min (~48 ± 5%) in rats treated with 5 mg/kg E217α (n = 6–8; P < 0.01). Administration of 6-ECDCA induced a dose-dependent improvement of cholestatic changes caused by E217α. At the dose 10 mg/kg/day (Fig. 3A; Table 2), it completely reversed cholestasis induced by E217α (n = 6–8; P < 0.01 versa...
FXR and Cholestasis

As shown in Fig. 4, 5-day administration of E217α (n = 6–8; P > 0.05 versus E217α) slightly increased liver Shp mRNA expression and reduced cyp7a1 and cyp8b1 mRNA by 40 to 70% (n = 4, P < 0.01 versus control) (Fig. 4, A–C). Furthermore, E217α treatment decreased ntcp and oatp1 mRNA expression by ~30 to 40% (n = 4; P < 0.01 versus control; Fig. 5, A and D), but it had no effect on bsep mRNA levels (n = 4; P > 0.05 versus control; Fig. 5G). Furthermore, E217α administration was associated with ~20% reduction of mdr2 mRNA (Fig. 5B), whereas it caused an ~8-fold increase in mdr3 gene expression (Fig. 5E), and 2- to 4-fold induction of mdr1a and mdr1b (n = 4; P < 0.05 versus control; Fig. 5, C and F). Administration of 6-ECDCA resulted in a 3- to 4-fold increase of Shp mRNA expression (n = 4; P < 0.05 versus control; Fig. 4C), which associates with a further decrease in cyp7a1 mRNA expression (n = 4; P < 0.01 versus control; Fig. 4A) and cyp8b1 (n = 4; P < 0.01 versus rats administered E217α; Fig. 4B) mRNA, the latter being reduced to almost undetectable levels. Consistent with Shp induction, treating rats with 6-ECDCA caused an ~80% reduction of ntcp mRNA (n = 4; P < 0.05 versus control; Fig. 5A) and increased bsep, mrp2, and mdr2 mRNA by 2- to 4-fold; n = 4; P > 0.05 versus control; Figs. 5, B, E, and H). Administering rats with 6-ECDCA reduced mdr1a mRNA expression, whereas it had no effect on induction of mdr3 and mdr1b caused by E217β (Fig. 5C). In contrast to 6-ECDCA, CDCA failed to up-regulate Shp mRNA expression over the effect of E217α (n = 4; P < 0.05 in comparison with control), resulting in a comparable inhibition of cyp7a1 and cyp8b1 mRNA expression in the liver (n = 4; P < 0.05 versus 6-ECDCA; Fig. 5). CDCA was also less effective than its derivative in regulating ntcp, bsep, mrp2, and mdr2 mRNA (n = 4; P > 0.05 versus E217α). As shown in Fig. 5, similarly to 6-ECDCA, CDCA had no effect on either mdr3, mdr1a, or mdr1b mRNA compared with E217α (n = 4; P > 0.05).

Confirming the protective role of FXR against cholestasis development, treating rats with GW4064 increased Shp expression by ~4-fold and reduced liver cyp7a1 and cyp8b1 mRNA by 60 to 70% (P < 0.05 versus E217α). GW4064 administration increased bsep, mrp2, and mdr2 mRNA expression by 2- to 4-fold (n = 4; P < 0.05 versus control), whereas similarly to 6-ECDCA it had no effect on mdr3, mdr1a, and mdr1b (P > 0.05 versus E217α). Finally, none of the treatments were effective in modulating oatp1 mRNA (n = 4; P > 0.05 versus E217α).
TABLE 2
Effect of natural and synthetic FXR ligands on bile acid output in E₂₁₇α-induced cholestasis
Numbers in parentheses indicate the percentage of each individual bile acid. Data are mean ± S.D. of 8 to 12 rats/group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>E₂₁₇α</th>
<th>E₂₁₇α + 6-ECDCA</th>
<th>E₂₁₇α + CDCA</th>
<th>E₂₁₇α + GW4064</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile flow (µL/kg min)</td>
<td>68.0 ± 6.0</td>
<td>33.3 ± 3.0*</td>
<td>66.0 ± 4.1**</td>
<td>39.0 ± 50.0*</td>
<td>71.0 ± 4.1**</td>
</tr>
<tr>
<td>Total bile acid output (µg/kg min)</td>
<td>187.5 ± 31.2</td>
<td>129.7 ± 35.3*</td>
<td>152.6 ± 28.3**</td>
<td>298.5 ± 239.9**</td>
<td>161.3 ± 17.3**</td>
</tr>
<tr>
<td>Individual bile salts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tauro-β-MCA</td>
<td>87.1 ± 11.6 (46.5%)</td>
<td>75.9 ± 16.4 (58.8%)</td>
<td>76.1 ± 14.1 (49.6%)</td>
<td>70.7 ± 54.9 (23.5%)</td>
<td>91.3 ± 10.8 (56.6%)</td>
</tr>
<tr>
<td>Taurocholic acid</td>
<td>77.1 ± 14.0 (41.1%)</td>
<td>42.3 ± 13.3* (32.0%)</td>
<td>33.1 ± 5.7* (23.7%)</td>
<td>63.0 ± 55.6 (21.3%)</td>
<td>47.0 ± 8.2 (29.1%)</td>
</tr>
<tr>
<td>TCDCA</td>
<td>8.7 ± 2.3 (4.6%)</td>
<td>7.3 ± 3.3 (5.6%)</td>
<td>9.3 ± 0.9 (6.4%)</td>
<td>101.1 ± 92.1* (34.2%)</td>
<td>14.1 ± 1.4 (8.7%)</td>
</tr>
<tr>
<td>TDCA</td>
<td>14.7 ± 3.3 (7.8%)</td>
<td>4.2 ± 2.3* (3.4%)</td>
<td>7.6 ± 1.7* (4.9%)</td>
<td>63.7 ± 37.3* (21.1%)</td>
<td>7.9 ± 1.4 (5.6%)</td>
</tr>
<tr>
<td>Tauro-6-ECDCA</td>
<td>N.D.</td>
<td>N.D.</td>
<td>26.5 ± 5.9* (17.4%)</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

N.D., not detected.

* P < 0.05 versus control; ** P < 0.05 versus E₂₁₇α.

Fig. 3. A–C, effects of 5-day administration of 6-ECDCA, CDCA, and GW4064 on bile flow in administered E₂₁₇α. Bile flow was measured as described under Materials and Methods after 5 days of administration of E₂₁₇α alone or in combination with 6-ECDCA, CDCA, or GW4064. Data are mean ± S.E. of six to eight rats per group. *, P < 0.05 versus control; **, P < 0.05 versus E₂₁₇α alone.
Discussion

In vitro studies in human and rodent hepatocytes have provided evidence that FXR regulates bile acid synthesis by modulating the activity of CYP7A1, the rate-limiting enzyme in the pathway involved in conversion of cholesterol into bile acids (Chiang, 2002). In the normal liver, liver X receptor (LXR), a nuclear receptor for oxysterols, acts as a cholesterol sensor and up-regulates the expression of CYP7A1 in response to increased levels of its ligands (Goodwin et al., 2000; Lu et al., 2000). FXR activation could provide additional signals such as production of an SHP ligand with the ability to confer SHP repression upon CYP7A1 and CYP8B1 promoters.

E2 administration is associated with a number of changes in the expression of basolateral and canalicular transporters (Fig. 6A). Thus, 5-day administration of E2 caused an ∼30 to 40% reduction of ntcp and oatp1 mRNA (Stieger et al., 1994; Trauner et al., 1998; Huang et al., 2000; Kullak-Ublick et al., 2003). Because ntcp is an important component of the sinusoidal systems that import bile acid into hepatocytes, and its expression is regulated by FXR-SHP regulatory cascade (Kullak-Ublick et al., 2003), a reduction of this transporter is likely to develop as an adaptation to represent cholestasis.

Our qRT-PCR analysis also demonstrates that the levels of mrp3, mdr1a, and mdr1b were increased in rats administered with E2. These canalicular transporters mediate efflux of xenobiotics and basolateral excretion of anion conjugates from hepatocytes. mdr1a and mdr1b expression is regulated by pregnane X receptor (Maglich et al., 2002; Goodwin et al., 2003). Since pregnane X receptor also is activated, among other ligands, by bile acids (Kast et al., 2002; Goodwin et al., 2003), induction of mdr1a and mdr1b is likely to represent an adaptive change that protects the liver by providing a mechanism for clearance of toxic constituents from hepatocytes.

Bile acids bind and activate FXR, with CDCA being the most active ligand (Forman et al., 1995; Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). Activation of FXR by endogenous ligand, however, requires micromolar concentrations, suggesting that development of potent FXR agonists, active in the nanomolar range, could help to activate this sensor mechanism by providing a mechanism for clearance of toxic constituents from hepatocytes. One potential risk of FXR agonists, however, might result from the feedback inhibition of bile acid synthesis, a mechanism that might cause a further impairment of cholestasis by reducing the biliary flow driven by bile salts. Our results, however, demonstrate that administration of potent FXR ligands protects against cholestasis development despite that it completely inhibited cyp7a1 and cyp8b1 mRNA expression. The

![Fig. 4. A–C, expression of cyp7a1, cyp8b1, and Shp mRNA after 5-day administration of 5 mg/kg E2 alone or in combination with 6-ECDA (10 mg/kg), CDCA (10 mg/kg), or GW4064 (20 mg/kg). Data are mean ± S.E. of four rats per group each assay carried out in triplicate.](https://aspetjournals.org/doi/10.1093/jpet/609.7.741)
The main explanation for this effect is that although CYP7A1 catalyzes the key, rate-limiting step of the classical, major pathway of the overall bile salt synthesis, bile acids can also be generated through an alternative pathway that involves the mitochondrial sterol 27-hydroxylase (CYP27A1) (Russell and Setchell, 1992). In contrast to the classical pathway that leads to synthesis of CA and deoxycholic acid (DCA), CYP27A1 catalyzes the first step in the alternative pathway, which produces selectively CDCA and the bile salts derived from its further hepatic and/or intestinal conversion, i.e., MCA (Russell and Setchell, 1992). CYP27A1 is not regulated by FXR, suggesting that bile acid synthesis through this pathway could be maintained even in the presence of FXR ligands. Consistent with this view, the analysis of individual bile acids in the bile of E$_{17a}$-treated rats (Table 2) demonstrated a relative enrichment of the bile salt pool by tauroMCA and TCDCA (i.e., bile acids generated through the alternative pathway) together with a decrease of CA and its secondary bile salt deoxycholic acid (Kern et al., 1977; Stieger et al., 1994; Koopen et al., 1999).

In the present study, we have demonstrated that a synthetic FXR ligand protects against development of cholestasis induced by estrogen administration to rats. At the dose of 10 mg/kg, 6-ECDCA profoundly inhibited Ntcp expression and caused, along with a 3-fold increase of Shp, a 2- to 3-fold increase in Bsep and Mrp2 and a 5-fold increase in Mdr2 mRNA. Since these basolateral (Ntcp) and canalicular (Bsep, Mrp2, and Mdr2) transporters are directly regulated by FXR (Denson et al., 2001) and their repression/induction is not observed or is only partially observed (Ntcp) in rats treated with E$_{17a}$ alone, their regulation is likely to play a mechanistic role in the anticholestatic effect of 6-ECDCA (Kullak-Ublick et al., 2003). Supporting this view, it has been shown that the cis- and trans-inhibition of Bsep is the main determinant of impaired bile flow in this model (Kullak-Ublick et al., 2003). BSEP and MRP2 are ATP-binding cassette proteins that transport bile acids and other organic anions across the canalicular membrane (Kullak-Ublick et al., 2003). Canicular transport of bile acids is a rate-limiting step in hepatic excretion of bile acids and BSEP and MRP2 are critically involved in this process. Mutation of BSEP and MRP2 genes in humans associated with cholestatic disorders (Trauner et al., 1998; Kullak-Ublick et al., 2003). Thus, inactive mutation of BSEP gene give rise to the type 2 progressive familial intrahepatic cholestasis (Strautnieks et al., 1998), whereas mutations of MRP2 give rise to Dubin-Johnson syndrome (Paulusma et al., 1997), a disease characterized by high serum bilirubin due to the inability of liver to excrete this metabolite into the bile. Induction of BSEP and MRP2 expression is under the control of FXR, and their modulation in response to CA feeding is lost in FXR null mice.
similarly to bile duct ligation and ECDCA administration increases mdr2/MDR3 expression by displacing more potent endogenous ligands from their binding site. In contrast to CDCA, bile induction caused by 6-ECDCA cannot be reverted in vitro by LCA (S. Fiorucci, unpublished data), suggesting that potent FXR ligands might overcome the effect of LCA in vivo.

In addition to bsep and mrp2, we have shown that 6-ECDCA administration increases mdr2/MDR3 expression by ~5-fold. Induction of mdr2/MDR3 by the FXR ligand GW4064 has recently been described in cholestasis induced by bile duct ligation and α-naphthyl-isothiocyanate (Liu et al., 2003). This canalicular transporter, the phospholipid flipase, mediates biliary secretion of phospholipids and cholesteryl ether, thus its induction by FXR ligand seems to be mechanistically involved in the anticholestatic effect induced by FXR ligands. Since 6-ECDCA is a CDCA derivative, there is a possibility that its effects are due to its conversion into CDCA. However, this seems not to be the case. Indeed, we found that T-CDCA concentrations did not increase in rats treated with 6-ECDCA, whereas a significant enrichment was observed in animals treated with CDCA alone, suggesting that 6-ECDCA is not converted to CDCA during its liver metabolism.

In conclusion, we have provided evidence that induction of the canalicular transporters bsep, mrp2, and mdr2/MDR3 and repression of bile acid biosynthesis mediate anticholestatic effects of 6-ECDCA. By demonstrating that 6-ECDCA protects against cholestasis induced in rat by estrogen administration, our data support the notion that development of potent FXR agonists might be beneficial in the treatment of cholestatic disorders.

References


Address correspondence to: Dr. Stefano Fiorucci, Gastroenterologia ed Epatoologia, Policlinico Monteluce, Via E dal Pozzo, 06111 Perugia, Italy. E-mail: fiorucci@unipg.it