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Full Length Article

Acetylated deoxycholic (DCA) and cholic (CA) acids are potent ligands of pregnane X (PXR) receptor

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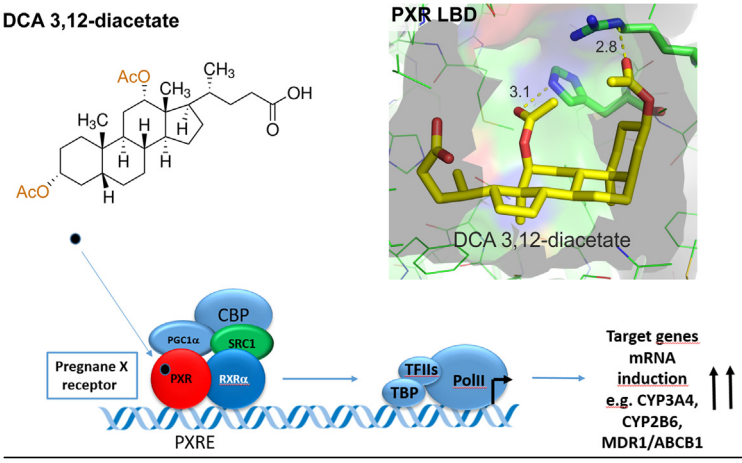
HIGHLIGHTS

- Acetylated deoxycholic (DCA) and cholic (CA) acids are potent ligands of PXR.
- Acetylated DCA and CA enhance PXR target genes expression.
- Dehydrogenation or acetylation of DCA, CA, lithocholic (LCA) or chenodeoxycholic (CDCA) do not lead to increased affinity to FXR or VDR.
- Acetylated DCA and CA were not found in bile.

GRAPHICAL ABSTRACT



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ABSTRACT

The Pregnane X (PXR), Vitamin D (VDR) and Farnesoid X (FXR) nuclear receptors have been shown to be receptors of bile acids controlling their detoxification or synthesis. Chenodeoxycholic (CDCA) and lithocholic (LCA) acids are ligands of FXR and VDR, respectively, whereas 3-keto and acetylated derivates of LCA have been described as ligands for all three receptors.

In this study, we hypothesized that oxidation or acetylation at position 3, 7 and 12 of bile acids DCA (deoxycholic acid), LCA, CA (cholic acid), and CDCA by detoxification enzymes or microbiome may have an effect on the interactions with bile acid nuclear receptors. We employed reporter gene assays in HepG2 cells, the TR-FRET assay with recombinant PXR and RT-PCR to study the effects of acetylated and

Abbreviations: BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; CYP, cytochrome P450; DCA, deoxycholic acid; 6-ECDCA, 6α-ethyl-chenodeoxycholic acid, obeticholic acid; FXR, farnesoid X receptor; LBD, ligand binding domain; LCA, lithocholic acid; PXR, pregnane X receptor; TR-FRET, time-resolved fluorescence energy transfer; VDR, vitamin D receptor.

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Nuclear receptors
FXR

keto bile acids on the nuclear receptors activation and their target gene expression in differentiated hepatic HepaRG cells.

We demonstrate that the DCA 3,12-diacetate and CA 3,7,12-triacetate derivatives are ligands of PXR and DCA 3,12-diacetate induces PXR target genes such as CYP3A4, CYP2B6 and ABCB1/MDR1.

In conclusion, we found that acetylated DCA and CA are potent ligands of PXR. Whether the acetylated bile acid derivatives are novel endogenous ligands of PXR with detoxification or physiological functions should be further studied in ongoing experiments.

1. Introduction

Three nuclear receptors of the nuclear receptor superfamily – the Pregnane X receptor (PXR, NR1I2), Farnesoid X (FXR, NR1H4) and Vitamin D receptor (VDR, NR1I1) – have been recently established as bile acid receptors in the liver and in the intestine and their role in bile acid (BA) synthesis regulation or detoxification has been clearly documented (Ishizawa et al., 2008; Makishima et al., 2002, 1999; Ridlon and Bajaj, 2015; Staudinger et al., 2001b; Wang et al., 1999; Xie et al., 2001). 3-Keto LCA has been found as a potent ligand for the VDR, FXR and PXR (Adachi et al., 2005; Makishima et al., 1999; Staudinger et al., 2001b), and the LCA acetate and LCA acetate methyl ester as highly potent VDR ligands (Adachi et al., 2005; Makishima et al., 1999; Staudinger et al., 2001b). It was also proposed that gut microbiome may produce endocrine molecules from steroids to activate nuclear receptors and several oxidized bile acid derivatives such as 7-oxo CA, 7-oxo DCA and 7-oxo CDCA have been identified as products of prokaryotic hydroxysteroid dehydrogenases (Ridlon and Bajaj, 2015).

We hypothesized that dehydrogenation (oxidation) or acetylation of bile acids may increase affinity to these nuclear receptors and that liver biotransformation enzymes or intestinal microflora may modify bile acids at position 3α, 7α and 12α of DCA, LCA, CA, and CDCA to derivatives more avidly interacting with the bile acid receptors (Ridlon and Bajaj, 2015). Therefore, to study structure-activity relationships (SAR) of dehydrogenated and acetylated bile acids with PXR, VDR and FXR receptors, we synthetized a series of dehydrogenated (keto) and acetylated derivatives of DCA, LCA, CA, and CDCA (Fig. 1). Some of these compounds are known products of gut microbiome bile salt 3α-, 7α, 12α-hydroxysteroid dehydrogenases or deconjugation enzymes as well as potential products of cytochrome P450-mediated biotransformation (compounds underlined in Fig. 1) (Deo and Bandiera, 2009; Ridlon and Bajaj, 2015; Ridlon et al., 2006).

The PXR has been identified as a “master” xenobiotic sensor regulating the expression of a wide variety of genes involved in the transport, metabolism and elimination of xenobiotics along with a number of endogenous substances. In addition, PXR has a function in regulating several cellular signaling pathways related to physiological processes (Banerjee et al., 2015). In the case of PXR, mainly lithocholic acid and its 3-keto derivative have been found to activate both human and mouse PXR (Krasowski et al., 2005; Staudinger et al., 2001b; Xie et al., 2001). 3-Keto LCA was found to be an even more potent ligand of PXR than LCA; whereas CDCA, DCA and CA only mildly activate PXR (Krasowski et al., 2005; Staudinger et al., 2001b). Therefore, PXR has been established as the receptor of LCA responsible for the detoxification of the highly hepatotoxic and a potentially enteric carcinogenic bile acid via induction of its metabolism (Staudinger et al., 2001b; Xie et al., 2001).

The FXR is localized mainly in the liver, intestine (ileum) and kidneys. FXR regulates the enterohepatic circulation and metabolism of bile acids, and it also modulates liver regeneration, inflammation and growth (Ali et al., 2015). Chenodeoxycholic acid (CDCA), and to a lesser extent lithocholic (LCA) and deoxycholic acid (DCA), are natural ligands of human FXR and able to transactivate the receptor, whereas cholic acid (CA) has weak effect on FXR activation (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). Among keto-bile acids, 7-keto and 3,7-keto LCA are known to activate FXR, although with lower potency (Wang et al., 1999). Conjugates of CDCA, LCA, and DCA with taurine and glycine and to a lesser extent CA conjugates can also activate FXR when transported into cells by a conjugate transporter such as ABST (IBAT, SLC10A2) (Makishima et al., 1999; Parks et al., 1999). Ligands of FXR are considered therapeutically useful for the treatment of liver disorders including various forms of cholestasis and fatty liver (steatosis) disease (Ali et al., 2015).

The VDR mediates the effect of the vitamin D active form 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$vitD_3). VDR is primarily associated with calcium and phosphate homeostasis, but it is also an important regulator of cell growth and differentiation, cell death and immunity (Dusso et al., 2005). It has been shown that VDR also functions as a receptor for the secondary bile acid lithocholic acid (LCA). VDR is the most sensitive receptor to activation by LCA and its metabolites in comparison with other nuclear receptors. Activation of VDR by LCA or by vitamin D induces expression of CYP3A4, a cytochrome P450 enzyme that detoxifies LCA in the liver and intestine (Makishima et al., 2002). Interestingly, derivatives of LCA such as 3-keto LCA (Makishima et al., 2002), LCA propionate and methylester LCA acetate display significantly higher affinity and potency to activate VDR (Adachi et al., 2005; Ishizawa et al., 2008).

In the current work, we attempted to determine if dehydrogenation or acetylation at position 3, 7 and 12 of unconjugated DCA, LCA, CA and CDCA has effects on interactions with the bile acid receptors PXR, FXR and VDR. We investigated interactions of synthetized acetylated, diacetylated, triacetylated and dehydrogenated bile acids CDCA, LCA, DCA and CA at positions 3 (α, β), 7α and 12α (Fig. 1) with PXR, FXR, and VDR nuclear receptors in a cellular assay as well as with recombinant nuclear receptor proteins in coactivator TR-FRET assays. Employing RT-PCR and *in silico* docking, we confirmed interactions of acetylated DCA and CA bile acids with PXR ligand binding domain (LBD) and PXR target genes regulation in HepaRG cells by DCA 3,12-diacetate. We further analyzed lipid extracts of mouse liver and human bile samples using HPLC/MS-MS.

2. Material and methods

2.1. Chemicals

The bile acid derivatives (Fig. 1) were synthesized at the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences. Synthesis procedures and NMR spectra are available upon request and will be published elsewhere.

2.2. Plasmids

The FXR response elements (FXRE)-driven luciferase reporter plasmid (pFXRE-luc2P) was constructed by inserting