European Journal of Pharmaceutical Sciences 123 (2018) 404–415

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

The ileum-liver Farnesoid X Receptor signaling axis mediates the compensatory mechanism of 17α-ethynylestradiol-induced cholestasis via increasing hepatic biosynthesis of chenodeoxycholic acids in rats

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ARTICLE INFO

Keywords:
17α-Ethynylestradiol
Chenodeoxycholic acids
Compensatory mechanism
Ileum-liver Fxr signaling axis
Intrahepatic cholestasis
Transporter
Enzyme

ABSTRACT

Background and purpose: Estrogen-induced intrahepatic cholestasis is one of the most common pathogenic factors for liver diseases in clinic. It is, however, regrettable that effective medical therapies to ameliorate or reverse this cholestasis are limited. Fortunately, the novel insights now allow us to target crucial transporters, enzymes and their regulatory pathways therapeutically by restoring disrupted bile acids (BAs) transport and signaling thus ameliorating cholestasis. Additionally, it has been found that a compensatory effect could have been developed under the condition of estrogen-induced in cholestasis. Hence, exploring the molecular mechanism of the adaptive changes counteracting the cholestasis would be one of the approaches for development of new therapeutic targets.

Methods: Parameters of BAs in different specimens, mRNA expressions of transporters, enzymes and farnesoid X receptor (Fxr) signaling pathways that relate to BAs homeostasis in liver and ileum were measured in rats with 7-day and 14-day 17α-ethynylestradiol (EE)-induced cholestasis, and the molecular docking and HepaRG cells studies for verification were also evaluated.

Key results: It has been found that the depression of "ileal Fxr-Fgf15 (fibroblast growth factor 15)-hepatic Cyp7a1 pathway" in coordinated with activation of "hepatic Fxr-Shp (small heterodimer partner)-Cyp8b1 pathway" as well as up-regulation of Cyp27a1 expression synergistically promoting the hepatic biosynthesis of chenodeoxycholic acids (CDCAs) that are the potent agonists of Fxr, contribute to the Bsep up-regulation mediated the bile flow restoration to alleviate the cholestasis.

Conclusion: These findings suggest that the adaptive regulation of Fxr-mediated ileum-liver signaling axis on Cyp7a1/Cyp8b1 might be the potentially novel targets for amelioration or treatment of estrogen-induced cholestasis, and we expect that this study would be of great value to provide a cue for patients with estrogen-induced cholestasis.

1. Introduction

Estrogen-induced intrahepatic cholestasis, frequently occurs in pregnant women, premenopausal women receiving oral contraceptives, postmenopausal women on hormone replacement therapy, or men receiving estrogen therapy for the treatment of prostate cancer, is one of the most common cholestasis in clinic (Li et al., 2016a; Reyes, 2016; Shi et al., 2014). It always originally results in liver damage followed by

Abbreviations: Asbt, apical sodium-dependent bile acids transporter; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BAs, bile acids; Bsep/BSEP, bile salt export pump; β-MCA, β-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; Cyp7a1, cholesterol 7α-hydroxylase cytochrome P450 7a1; Cyp8b1, sterol 12-alpha-hydroxylase; Cyp27a1, sterol 27-hydroxylase; DBIL, direct/conjugated bilirubin; DCA, deoxycholic acid; EE, 17α-ethynylestradiol; Fxr/FXR, farnesoid X receptor; Fgf15/FGF19, fibroblast growth factor 15/19; G-, glycine conjugated bile acid; HDCA, hyodesoxycholic acid; ICP, intrahepatic cholestasis of pregnancy; LCA, lithocholic acid; IBIL, indirect/unconjugated bilirubin; NDCA, demethylation deoxycholic acid; Ntcp, Na^+-taurocholate co-transporting polypeptide; Ostα/β, heterodimeric organic solute transporters alpha and beta; Shp, small heterodimer partner; T-, taurine conjugated bile acid; TBIL, total bilirubin; UDCA, ursodeoxycholic acid; 6E-CDCA, 6α-ethyl-chenodeoxycholic acid

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https://doi.org/10.1016/j.ejps.2018.08.005
Received 30 November 2017; Received in revised form 19 June 2018; Accepted 1 August 2018
Available online 02 August 2018

biliary fibrosis, cirrhosis and finally end-stage liver disease (Reyes, 2016; Wikstrom Shemer et al., 2015). It is, however, regrettable that effective medical therapies to ameliorate or reverse the estrogen-induced cholestasis are limited. Ursodeoxycholic acid (UDCA) is the only FDA approved drug for treatment of cholestasis at present, but its therapeutic effect is not very well (Marschall et al., 2005). In addition, and perhaps most importantly, it has been found in our study that the symptoms of intrahepatic cholestasis especially the decreased bile flow were restored after 14 days administrating with 17α-ethynylestradiol (EE), a synthetic estrogen and a key ingredient of many oral contraceptives commonly applies to imitate the estrogen-related cholestasis in rodents (Marrone et al., 2016; Rodriguez-Garay, 2003). This result was similar to the previous study from John M. Lee et al., indicating bile acids (BAs) excretion was persisted following chronic cholestasis in the rats (Lee et al., 2000). With this background, it is proposed that compensatory response exist in the context of estrogen-induced intrahepatic cholestasis, but the underlying mechanism still remain largely uncharacterized. Herein, it may be worthwhile to explore the compensatory mechanism, which would contribute to identify and develop novel therapeutic targets for pharmacological interventions of estrogen-induced intrahepatic cholestasis.

Intrahepatic cholestasis, refers to the impairment of bile formation and bile flow, should fundamentally be ascribed to the disorder of enzymes and transporters systems associating with the BAs homeostasis (Gonzalez-Sanchez et al., 2015; Thakkar et al., 2017). More specifically, BAs synthesis can be accomplished via being catalyzed by enzymes in the classical (or neutral) pathway and the alternative (or acidic) pathway in liver. In the classical pathway, it has been well established that the rate-limiting enzyme cholesterol 7α-hydroxylase (Cyp7a1) initiates biosynthesis of primary BAs including cholic acid (CA) and chenodeoxycholic acid (CDCA), and sterol 12α-hydroxylase (Cyp8b1) that is required for CA synthesis determines the proportion of CA and CDCA in BAs pool. In the alternative pathway, sterol 27-hydroxylase (Cyp27a1) is the essential enzyme just for CDCA hepatic generation (Brown and Sharpe, 2016), and it also play a role in determining the proportion of CA and CDCA in vivo. Importantly, hepatobiliary transports systems play an important role in determining BAs enterohepatic circulation (Keppler, 2017). At distal ileum, the pivotal position for BAs reabsorption, approximately 95% BAs reabsorb into enterocytes by apical sodium-dependent BAs transporter (Asbt, *Slc10a2*), and then secrete into the portal circulation by basolateral heterodimeric organic solute transporters alpha and beta (Ostα/β, *Slc51a/Slc51b*). Most of the portal BAs (> 95%) uptake into hepatocytes by hepatic sinusoidal transporters Na^+-taurocholate cotransporting polypeptide (Ntcp, *Slc10a1*) and efflux into bile by bile salt export pump (Bsep, *Abcb11*) at canalicular membrane (Dawson, 2016). Additionally and even more significantly, BAs are the natural ligands of farnesoid X receptor (Fxr, gene symbol *Nr1h4*), and they can active Fxr signaling pathways including fibroblast growth factor 15 (Fgf15, orthologous as human FGF19) and small heterodimer partner (Shp, *Nr0b2*) in ileum and liver to coordinately regulate genes encoding for the BAs-related enzymes and transporters mentioned above at transcriptional level. Altogether, the Fxr signaling pathways, enzymes and transporters systems in ileum and liver as underlined above constituting the "ileum-liver Fxr signaling axis" mediates the BAs homeostasis in vivo (de Aguiar Vallim et al., 2013; Wagner et al., 2011), and it is originally thought that the compensatory mechanism of EE-induced intrahepatic cholestasis could be associated with adaptive alteration of the BAs as well as the ileum-liver Fxr signaling axis. The details of "ileum-liver Fxr signaling axis" are descripted in Fig. 1.

In short, the main aim of this study is to elucidate the compensatory mechanism of EE-induced intrahepatic cholestasis via the "ileum-liver Fxr signaling axis" in rats. To address this question, in the present work, the alteration of BAs concentrations and compositions in liver and ileum and the adaptive regulation of "ileum-liver Fxr signaling axis" including Fxr-signaling pathways, enzymes and transporters mentioned above were investigated under the condition of EE-induced cholestasis for 7 and 14 days in rats, respectively. Furthermore, the molecular docking study was used to verify the hypothesis about the pathogenetic and compensatory mechanism of EE-induced cholestasis, and the associated conclusion was further verified in human HepaRG cells, which maintain drug-metabolizing enzymes, hepatobiliary transporters and nuclear receptors similar to those in primary human hepatocytes and is considered optimal for evaluating hepatotoxicity as a reliable surrogate of primary human hepatocytes (Marion et al., 2010; Tolosa et al., 2016). We expect the current study provides a valuable cue for the further studies on identifying novel and effective therapeutic targets for amelioration or treatment of estrogen-induced cholestatic disorders.

2. Materials and methods

2.1. Animals and treatment

Adult male Wistar rats with body weight around 220 g were obtained from the Laboratory Animal Center of Lanzhou University (Lanzhou, China). The rats were housed in a standard laboratory with a temperature and humidity-controlled environment under a constant 12:12-hour light/dark cycle, and diet and water ad libitum. Animal experiments were implemented according to the guidelines of the Lanzhou University Ethics Review Committee, and were approved by the Ethical Committee for Animal Experiments of the Lanzhou University. In the current study, the cholestatic rats model were induced by the classical method of subcutaneously administration with EE (Lee and Boyer, 2000; Marrone et al., 2016; Zollner and Trauner, 2006). All rats were randomly divided into four experimental groups (n = 10 per group): rats in 7-day EE group were subcutaneously administered with EE at a daily dose of 5 mg/(kg body weight) for 7 consecutive days; rats in 14-day EE group were administered subcutaneously with 5 mg/(kg body weight) dose of EE for first 7 consecutive days and 3 mg/(kg body weight) maintenance dose for the next 7 consecutive days (Hung et al., 2005); rats in 7-day control group and 14-day control group received only the propylene glycol (the vehicle of EE) for 7 and 14 days, respectively. The data about EE level in serum and liver of rats in different groups was described in Supplemental data (Supplementary Fig. 1).

2.2. Samples collection

After intervention accomplishment, 5 rats were randomly selected from each group, and the 12-hour feces were collected from 10 PM to 10 AM of the second day, during this time, animals were fasting. After that, rats were anesthetized by intraperitoneal injection of urethane (500 mg/kg body weight), and then a middle abdominal incision was made and the common bile duct was cannulated with PE-10 polyethylene tube for bile collection. Bile was collected for 1 h and bile flow was determined by gravimetry, assuming a density of the bile of 1.0 g/mL. At the end of bile collection, animals were euthanized with an overdose of anaesthesia. In addition, blood, liver and ileum samples were usually obtained from the anesthetic rats without bile collection after 12 h of fasting at 10 AM (n = 5), and blood samples were collected from abdominal aorta. The samples for BAs analysis by HPLC/MS/MS and mRNA expression determination by real-time quantitative PCR (RT-qPCR) were respectively frozen in −80 °C and in liquid nitrogen.

2.3. Evaluation of serum transaminase, bilirubin and total BAs

The level of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin that include total bilirubin (TBIL), conjugated bilirubin (direct bilirubin, DBIL) and unconjugated bilirubin (indirect bilirubin, IBIL) in serum were measured using the Chemistry Analyzer (OLYMPUS AU400, Tokyo, Japan) in Department of Infection in The First Hospital of Lanzhou University.