Digestive and Liver Disease 48 (2016) 771–779

Contents lists available at ScienceDirect

Digestive and Liver Disease

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

Liver, Pancreas and Biliary Tract

Glucocorticoid treatment alters systemic bile acid homeostasis by regulating the biosynthesis and transport of bile salts

Yongtao Xiao[a,b,c], Weihui Yan[a,c], Kejun Zhou[b,c], Yi Cao[a], Wei Cai[a,b,c,*]

[a] Department of Pediatric Surgery, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[b] Shanghai Institute for Pediatric Research, Shanghai, China
[c] Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai, China

ARTICLE INFO

Article history:
Received 15 February 2016
Accepted 23 March 2016
Available online 11 April 2016

Keywords:
Bile acid
Biliary atresia
Glucocorticoid receptor
Glucocorticoids
Homeostasis

ABSTRACT

Background: Dysregulation of systemic bile acid homeostasis can lead to cholestatic liver diseases and metabolic syndromes. As important anti-inflammatory and immunosuppressive drugs, synthetic glucocorticoids (GCs) are used to treat several cholestatic disorders, including biliary atresia (BA), because of their effects on the regulation of bile acid metabolism. However, the molecular mechanisms that underlie GCs regulation of bile acid homeostasis remain unclear.
Aims: To provide a mechanistic basis for the effects of GCs on bile acid homeostasis.
Methods: Male rats were treated with methylprednisolone for 7 days with slow-release osmotic pumps under physiological and cholestatic status that was induced by bile duct ligation (BDL). Expression of glucocorticoid receptor (GR) and genes related to bile acid metabolism was investigated using western blotting, qRT-PCR and immunohistochemistry.
Results: We show here that sustained treatment with GCs in rats disrupts the normal changes in systemic bile acid distribution by elevating plasma bile acid levels and reducing faecal bile acid loss. Treatment with GCs stimulated bile acid absorption in the ileum by increasing expression of the apical sodium-dependent bile acid transporter (Asbt). Concomitantly, administration of GCs enhanced liver bile acid uptake by increasing the expression of the major hepatocyte basolateral bile transporter (Ntcp). The reduced expression of a bile acid synthesis rate-controlling enzyme, Cyp7a1, suggests that treatment with GCs suppressed hepatic bile acid synthesis.
Conclusion: Our study provides evidence that GCs can increase enterohepatic bile acid circulation through regulation of the biosynthesis and transport of bile salts, which suggests that plasma bile acid levels should be monitored during treatment with GCs in patients with BA.

1. Introduction

Systemic bile acid homeostasis is regulated by extensive cross-talk between the liver and small intestine. During the enterohepatic cycling of bile acid, 95% of bile acid released from the liver into the intestine is reabsorbed by the distal ileum and transported back to the liver through portal circulation. The 5% of bile acid that is unabsorbed is eliminated in the faeces and will be supplemented by novel hepatic synthesis [1]. The dysregulation of bile acids has been associated with a number of gastrointestinal and metabolic diseases [2,3]. However, the molecular mechanisms that regulate bile acid homeostasis have not been fully elucidated.

Glucocorticoids (GCs) are released from the adrenal gland under control of the hypothalamic-pituitary-adrenal (HPA) endocrine axis. Synthetic glucocorticoids have well-known effects on the immune response and are widely used as anti-inflammatory and immunosuppressive drugs. Recently, it has been shown that GCs can be used in treating several cholestatic disorders because of their effects on the regulation of bile acid metabolism [4–6]. Cholestatic disorders are characterized by the accumulation of toxic bile acid in the liver and the systemic circulation, a typical feature of human cholestatic liver disease, such as biliary atresia (BA) in children [7]. Although GCs have been used to treat BA empirically for many years, there is no consensus on their benefits for improving the outcomes following Kasai portoenterostomy [8–10]. Thus, investigation of the precise molecular mechanisms by which GCs influence the outcomes of Kasai portoenterostomy is needed.

* Corresponding author at: Department of Pediatric Surgery, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.
Tel.: +86 21 25076449; fax: +86 21 65791316.
E-mail address: caiw1978@163.com (W. Cai).

http://dx.doi.org/10.1016/j.dld.2016.03.022
1590-8658/

The function of GCs is mediated by the glucocorticoid receptor (GR), which regulates a number of transcriptional programs [11]. GCs bind to the GR and activate it by dissociating receptor-bound heat shock proteins into the cytoplasm. Activated GR translocates to the nucleus and binds directly to the GR response element (GRE) in the promoter regions of down-stream target genes, such as genes related to bile acid biosynthesis and transport [12,13]. Indeed, Rose et al. reported that absence of the hepatic GR in mice substantially enhanced faecal loss and led to a decrease in gallbladder bile acids [14]. In humans, prednisolone treatment elevated plasma bile acid levels in patients with chronic active hepatitis [15]. Recently, possible molecular mechanisms of how GCs might influence bile acid homeostasis have been described. With regard to bile acid transport, it has been demonstrated that treatment with GCs increases the expression of the major hepatocyte basolateral bile acid transporter, Na+-taurocholate transport protein (Ntcp/Slc10a1), in a GR-dependent manner [14,16]. Out et al. showed that Asbt (Slc10a2), the main intestinal bile acid transporter localized in the terminal ileum, was induced upon prednisolone treatment [17]. Bile acid is synthesized from cholesterol by several enzymes, of which the cytochrome P450, family 7, subfamily a, polypeptide 1 (Cyp7A1) is a rate-controlling enzyme. The expression of Cyp7A1 is regulated by multiple factors, including glucose, insulin, bile acid and glucocorticoid hormones [18,19]. Mitropoulos et al. showed that adrenalectomy resulted in a significant loss of Cyp7A1 expression leading to an inhibition of bile acid synthesis that can be restored by treatment with cortisol [20]. In addition, Cyp7A1 expression and activity follow a robust diurnal rhythm, and the changes in hepatic Cyp7A1 mRNA expression correlate with serum corticosterone levels [21].

In this study, we sought to investigate the effects and mechanisms of synthetic GCs on the physiology of bile acids *in vivo* and *in vitro*. We show that sustained treatment of rats with GCs increases the enterohepatic cycling of bile acid by increasing intestinal bile reabsorption and hepatic bile acid uptake, leading to elevated plasma bile acid levels and decreased faecal bile acid loss.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum (FBS) were purchased from GIBCO (Los Angeles, CA); Anti-Foxa2 was obtained from Abcam (NT, HK); Anti-SLC10A2 (ASBT) was purchased from Sigma Life Science (Louis, MO); Anti-SLC10A1 (NTCP) was purchased from Santa Cruz (Santa Cruz, CA); Anti-Cyp7a1 was obtained from Millipore (Darmstadt, Germany); Anti-Glucocorticoid Receptor alpha was obtained from Novus (Littleton, CO); Anti-GAPDH was obtained from Cell Signaling (Danvers, MA); Mini-osmotic pumps were purchased from ALZET (Cupertino CA). Kits used in this study included SYBR-Green Universal Master Mix kit and a High Capacity cDNA Reverse Transcription kit (Applied Biosystems Foster City, CA), as well as the Dual Luciferase Assay Kit (Promega, Madison, WI). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA).

2.2. Animal experiments

Approximately 4-week-old male Sprague-Dawley rats were housed individually in a temperature- and light-controlled facility with 12 h light-dark cycling. Slow-release methylprednisolone mini-osmotic pumps (ALZET, Cupertino CA) with a calculated release of 20 mg/kg/day were implanted subcutaneously for 7 days. Rats ($n = 9$) were subjected to a bile duct ligation (BDL) operation ($n = 9$) or BDL plus methylprednisolone (mini-osmotic pumps, 2 mg/kg/day, $n = 8$) treatment for 7 days as previously described [14–16]. All animal experimental protocols were approved by the Shanghai Jiao Tong University School of Medicine affiliated Xin Hua hospital Animal Care and Use Committee.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using Trizol according to the manufacturer's protocol (Invitrogen, Foster, CA). A SYBR-Green Universal Master Mix kit and a High Capacity cDNA Reverse Transcription kit were employed to detect the gene expression levels. The primers are listed in Supplemental Table 1.

2.4. Western blots

Western blots were performed as previously described [19]. Antibodies to Cyp7a1, NTCP, FOXA2 and GAPDH were used.

2.5. Immunohistochemistry

Immunohistochemistry (IHC) analysis was performed as previously described [21]. Antibodies for Cyp7a1, GR, FOXA2, NTCP and ASBT were used in this study.

2.6. Biochemical measurements

The plasma biochemistries for all animals were analyzed with a CHEMIX-180 multiple analyser (Sysmex Corporation, Japan) as previously described [22]. Bile acids from the plasma, liver and faeces were measured according to previously reported methods [23,24]. The detection was performed using Waters ACQUITY ultra performance liquid chromatography (BEH C18 1.7 μm 2.1 × 100 mm column) coupled with Waters Xevo TQ-S triple quadruple mass spectrometry. Data acquisition and quantification of bile acids were performed using MassLynx 4.1 software (Waters).

2.7. Cell culture and transfection

Hepatocarcinoma (Huh-7) cells and colon carcinoma (Caco2) cells were purchased from the American Type Culture Collection (ATCC) and were grown in a 37 °C incubator maintained at 5% CO_2. The siRNAs against FOXA2 and an irrelevant 21-nucleotide control siRNA (Negative Control) were purchased from GenePharma Biotechnology (Shanghai, China). pcDNA3-FOXA2 and pcDNA3-GR were obtained from Genechem Biotechnology (Shanghai, China). Transfections were carried out using Lipofectamine 2000 (Life technologies, Carlsbad, CA).

2.8. Promoters of luciferase reporter vectors

To construct reporter vectors carrying the promoters of CYP7A1, SLC10A1 and SLC10A2, we synthesized the promoter fragments for human CYP7A1 (1198 nt), SLC10A1 (1257 nt) and SLC10A2 (1490 nt) and cloned them into the psiCHECK2 luciferase vector (Promega, Madison, WI). For mutant vectors, the GR binding elements were mutated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA).

2.9. Dual luciferase reporter assay

Transient transfection of Huh-7 cells with pcDNA3-FOXA2, pcDNA3-GR, and FOXA2 siRNAs and the promoters of CYP7A1, SLC10A1 or SLC10A2 was carried out using Lipofectamine 2000. After 24 h, medium containing 5 μM dexamethasone (Dex) was added. Forty hours later, cells were harvested and washed with phosphate buffered saline. Cell lysates were assayed for luciferase