Biochemical Pharmacology 91 (2014) 483–489

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

All-trans retinoic acid regulates hepatic bile acid homeostasis

Fan Yang [a,b], Yuqi He [b], Hui-Xin Liu [b], Jessica Tsuei [b], Xiaoyue Jiang [c], Li Yang [a], Zheng-Tao Wang [a], Yu-Jui Yvonne Wan [a,b,*]

[a] Institute of Chinese Material Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cai-Lun Road, Shanghai 201203, China
[b] Department of Pathology and Laboratory Medicine, the University of California at Davis Medical Center, 4645, 2nd Avenue, Sacramento, CA 95817, USA
[c] Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134, USA

ARTICLE INFO

Article history:
Received 17 July 2014
Accepted 19 August 2014
Available online 28 August 2014

Chemical compounds studied in this article:
All-trans retinoic acid (Pubchem CID: 444795)

Keywords:
Liver
Nuclear receptor
FXR
SHP
CYP7A1
CYP8B1

ABSTRACT

Retinoic acid (RA) and bile acids share common roles in regulating lipid homeostasis and insulin sensitivity. In addition, the receptor for RA (retinoid x receptor) is a permissive partner of the receptor for bile acids, farnesoid x receptor (FXR/NR1H4). Thus, RA can activate the FXR-mediated pathway as well. The current study was designed to understand the effect of all-trans RA on bile acid homeostasis. Mice were fed an all-trans RA-supplemented diet and the expression of 46 genes that participate in regulating bile acid homeostasis was studied. The data showed that all-trans RA has a profound effect in regulating genes involved in synthesis and transport of bile acids. All-trans RA treatment reduced the gene expression levels of *Cyp7a1*, *Cyp8b1*, and *Akr1d1*, which are involved in bile acid synthesis. All-trans RA also decreased the hepatic mRNA levels of *Lrh-1* (*Nr5a2*) and *Hnf4α* (*Nr2a1*), which positively regulate the gene expression of *Cyp7a1* and *Cyp8b1*. Moreover, all-trans RA induced the gene expression levels of negative regulators of bile acid synthesis including hepatic *Fgfr4*, *Fxr*, and *Shp* (*Nr0b2*) as well as ileal *Fgf15*. All-trans RA also decreased the expression of *Abcb11* and *Slc51b*, which have a role in bile acid transport. Consistently, all-trans RA reduced hepatic bile acid levels and the ratio of CA/CDCA, as demonstrated by liquid chromatography-mass spectrometry. The data suggest that all-trans RA-induced SHP may contribute to the inhibition of CYP7A1 and CYP8B1, which in turn reduces bile acid synthesis and affects lipid absorption in the gastrointestinal tract.

1. Introduction

The role of retinoids in lipid metabolism is an emerging field in retinoid research due to their ability to regulate lipid homeostatic processes including adipogenesis, lipolysis, and fatty acid oxidation. Natural and synthetic retinoids are promising treatments for metabolic syndrome, obesity, and diabetes mellitus [1–5]. It has been shown that all-trans retinoic acid (all-trans RA), the active metabolite of vitamin A, can reduce body fat and improve insulin sensitivity in both lean and obese rodents [6]. Similarly, there is an inverse relationship between vitamin A intake and adiposity in healthy people [7]. Moreover, using genome-wide approaches, we showed that all-trans RA reduces serum lipid *in vivo* [8].

While there are beneficial effects of retinoids, published data also demonstrated their negative impact in human patients. For example, body weight gain and hyperlipidemia are common side effects when all-trans RA is used to treat acute promyelocytic leukemia [9,10]. Likewise, hypertriglyceridemia is common in patients who receive retinoid treatment for dermatological disorders [11]. The controversial findings may be explained, in part, by the models used. The conflicting effects of RA treatments may also be due to the fact that retinoid x receptor (RXR), the RA receptor, is a permissive or active partner for the receptors of fatty acids (peroxisome proliferator activated receptors), oxysterols (liver x receptors), and bile acids (farnesoid x receptor) [12]. These receptors regulate lipid homeostasis and often have opposing effects. For example, PPARα (NR1C1), which is highly expressed in the liver, is responsible for lipid oxidation, while PPARγ (NR1C3) is involved in lipogenesis [13]. Thus, RA-mediated activation of one pathway can result in deactivation of another in a tissue-specific manner. Taken together, the role of retinoids in lipid homeostasis is complicated and warrants further investigation.

* Corresponding author.
E-mail addresses: fanyangforever@gmail.com (F. Yang), hyqjeff@gmail.com (Y. He), huixinliu2012@gmail.com (H.-X. Liu), Jltsuei10@gmail.com (J. Tsuei), xiaoyue.jiang@thermofisher.com (X. Jiang), yangli7951@hotmail.com (L. Yang), wangzht@hotmail.com (Z.-T. Wang), yjywan@ucdavis.edu (Y.-J. Wan).

http://dx.doi.org/10.1016/j.bcp.2014.08.018
0006-2952/

Bile acids are responsible for lipid absorption in the gastrointestinal tract. They are synthesized in the liver by cholesterol catabolism *via* the classical (CYP7A1 and CYP8B1) and the acidic pathways (CYP27A1 and CYP7B1) [14]. Upon ingestion of fat and protein, intestinal cholecystokinin stimulates the release of bile containing digestive enzymes and primary bile acids from the gallbladder [15]. In the liver, bile acids activate FXR, which induces the expression of SHP to inhibit the activity of LRH-1 and HNF4α, which are the positive regulators of *Cyp7a1* [16,17]. In the small intestine, bile acid-activated FXR can induce the expression of fibroblast growth factor 15/19 (FGF15/19, rodents/humans), which binds hepatic FGFR4 and activates ERK1/2 (MAPK1/3) and JNK1/2 (MAPK8/9) to inhibit hepatic bile acid synthesis [18–22]. Bile acids are actively reabsorbed from the ileum by IBAT (SLC10A2) and circulate back to the liver through the hepatic portal vein [23]. This highly efficient process ensures that the majority of bile acids are recycled [24]. SLC51A/B (OSTα/β) a heteromeric complex, functions as a major basolateral transporter of bile acids [25], and is essential for intestinal bile acid transportation as well [26]. FXR not only regulates bile acid homeostasis, it also has a role in controlling insulin resistance and glucose homeostasis [27]. Since both RA and bile acids regulate lipid homeostasis and insulin sensitivity, the current study examines the pharmacological effect of all-trans RA on bile acid homeostasis.

Using non-biased approaches, we have conducted microarray study, bile acid quantification, and proteomic study to analyze the action of all-trans RA in bile acid homeostasis. Our data show that all-trans RA has a significant effect in regulating bile acid homeostasis *in vivo*. All-trans RA inhibits bile acid synthesis by down regulating the expression of key bile acid synthesis enzymes, CYP7A1 and CYP8B1. All-trans RA also modulates the expression of genes that have a role in the transportation of bile acids. All-trans RA treatment reduced the CA/CDCA ratio and may have an impact on lipid absorption.

2. Materials and methods

2.1. In vivo *and* in vitro *experiments*

Wild type C57/BL mice were purchased from the Jackson Laboratory (Sacramento, CA, USA) and Slac Laboratory Animal (Shanghai, China). Mice were treated with standard rodent chow or supplemented with all-trans RA (Sigma, St. Louis, MO) (150 mg/kg diet) for seven days. The concentration was chosen due to its anti-carcinogenic effect in a rat model of liver carcinogenesis in the absence of severe toxicity [28]. At the end of the treatment, mice were anesthetized with isoflurane and euthanized. Livers and ileums were frozen in liquid nitrogen immediately after collection and stored in −80 °C freezer for further assays. The animal treatment experiment was repeated twice using sample size of 5 per control and all-trans RA-treated group. Animal protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kansas Medical Center and the University of California, Davis as well as the Experimental Animal Ethics Committee at the Shanghai University of Traditional Chinese Medicine.

Primary human hepatocytes, derived from two donors, were provided by XenoTech (Lenexa, KS). Cells were treated with DMSO and all-trans RA (10 μM) for 12 h in triplicates followed by RNA extraction to study gene expression.

2.2. Quantification of bile acids

An ultra-performance liquid chromatography instrument coupled with a quatropole mass spectrometry (UPLC–MS, Waters Co., MA, USA) was used to detect hepatic bile acids. Livers were homogenized in acetonitrile (100 mg tissue/500 μl acetonitrile) followed by centrifugation at 14,300 rpm for 10 min. The supernatant was dried under nitrogen steam, then re-dissolved in methanol solution (methanol:water:formic acid = 50:50:0.01) followed by centrifugation at 14,300 rpm for 10 min. The supernatants were injected into the UPLC–MS instrument. Both the UPLC and MS parameters were described in our previous publication [29]. Bile acids profiles in control and all-trans RA-treated mice were differentiated by principle component analysis (PCA). Significance of differences between individual bile acid levels in control and all-trans RA-treated mice were examined by student's *t*-test.

2.3. Global expression profile of genes that contribute to bile acid homeostasis in control and all-trans RA-treated mice

The hepatic gene expressions in control and all-trans RA-treated mice ($n = 3$) were studied by microarray as described in our previous publication [30]. Microarray data are available in the public database (NIH GEO, GSE50028). Bile acid-related genes ($n = 46$) were selected from the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database. Expression levels of these 46 genes were extracted from the microarray data. PCA was used to describe the expression profile of these genes in control and all-trans RA-treated groups. student's *t*-test was used to differentiate expression profiles between groups based on PCA scores.

2.4. Proteomics

Proteins were extracted from mouse livers by T-PER (Thermo Scientific, Rockford, IL, USA). Protein concentrations were determined by protein assay kit (Thermo Scientific, Rockford, IL, USA). Proteins (200 μg) were precipitated and washed with protein precipitation kit (Calbiochem, La Jolla, CA, USA). The protein pellets were reconstituted in urea solution (6 M in 25 mM ammonium bicarbonate) and then mixed with reducing reagent containing 30 mg/ml DTT in 25 mM ammonium bicarbonate. After 1 h incubation at room temperature, 20 μl iodoacetamide (200 mM in 25 mM ammonium bicarbonate) was added for alkylation, which was quenched by dilution with 10 times the volume of ammonium bicarbonate (25 mM). The diluted samples were mixed with 10 μl trypsin (0.7 mg/ml in 25 mM ammonium bicarbonate), then incubated overnight at 37 °C for digestion. The digested samples were cleaned with Aspire RP30 tips (Thermo Scientific, Rockford, IL, USA) followed by injection into the nano-liquid chromatography (nLC) coupled with MS instrument (Q-Exactive, Thermo Fisher, San Jose, CA, USA). The nLC–MS parameters as well as the data processing and annotation were performed based on previously published methods [31]. PCA was used to describe the global expression profile of the mouse hepatic proteome in control and all-trans RA-treated groups. A volcano plot was generated to visualize both fold change and significance of the protein expression levels between control and all-trans RA-treated mice.

2.5. Quantification of mRNA

Total RNA was extracted using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) followed by reverse transcription using the high capacity cDNA reverse transcription kits (Applied Biosystems, Foster City, CA, USA). The mRNA levels were quantified by real-time PCR on an ABI 7900HT fast real-time PCR system (Applied Biosystems, CA, USA) using Power SYBR Green PCR master mix (Applied Biosystems, CA, USA). Hepatic and ileal mRNA levels were normalized to the mRNA level of glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*). *Gapdh* is considered a house-keeping gene and frequently used as a reference gene [32,33]. In addition, it has been shown that *Gapdh* is one of the most stable reference