The International Journal of Biochemistry & Cell Biology 44 (2012) 2019–2027

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Induction of carnitine palmitoyl transferase 1 and fatty acid oxidation by retinoic acid in HepG2 cells

Jaume Amengual[a,b], Petar Petrov[a], M. Luisa Bonet[a], Joan Ribot[a,*], Andreu Palou[a]

[a] Laboratory of Molecular Biology, Nutrition and Biotechnology, Universitat de les Illes Balears (UIB), Palma de Mallorca and CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn), Spain
[b] Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form 24 July 2012
Accepted 25 July 2012
Available online 31 July 2012

Keywords:
Vitamin A
Retinoic acid
Liver lipid metabolism
Fatty acid oxidation
Fatty liver
Rxr

ABSTRACT

The vitamin A derivative retinoic acid (RA) is an important regulator of mammalian adiposity and lipid metabolism, primarily acting at the gene expression level through nuclear receptors of the RA receptor (RAR) and retinoid X receptor (RXR) subfamilies. Here, we studied cell-autonomous effects of RA on fatty acid metabolism, particularly fatty acid oxidation, in human hepatoma HepG2 cells. Exposure to all-*trans* RA (ATRA) up-regulated the expression of carnitine palmitoyl transferase-1 (CPT1-L) in HepG2 cells in a dose- and time-dependent manner, and increased cellular oxidation rate of exogenously added radiolabeled palmitate. The effect of ATRA on gene expression of CPT1-L was: dependent on ongoing transcription, reproduced by both 9-*cis* RA and a pan-RXR agonist (but not a pan-RAR agonist) and abolished following RXRα partial siRNA-mediated silencing. CPT1-L gene expression was synergistically induced in HepG2 cells simultaneously exposed to ATRA and a selective peroxisome proliferator-activated receptor α agonist. We conclude that ATRA treatment enhances fatty acid catabolism in hepatocytes through RXR-mediated mechanisms that likely involve the transactivation of the PPARα:RXR heterodimer. Knowledge of agents and nutrient-derivatives capable of enhancing substrate oxidation systemically and specifically in liver, and their mechanisms of action, may contribute to new avenues of prevention and treatment of fatty liver, obesity and other metabolic syndrome-related disorders.

1. Introduction

Changes in hepatic lipid metabolism including defective mitochondrial fatty acid oxidation or increased lipogenesis can account for excessive hepatic triacylglycerol accumulation. Non-alcoholic hepatic steatosis is the main cause of abnormal liver function, closely relates to insulin resistance, and can further progress to non-alcoholic steatohepatitis, fibrosis, cirrhosis and even hepatocellular carcinoma (Kotronen and Yki-Jarvinen, 2008; Vanni et al., 2010). Hepatic lipid metabolism is influenced by a complex system of nutritional factors, hormones, signaling pathways and transcription factors. The peroxisome proliferator-activated receptor α (PPARα) and the liver X receptor (LXR) – sterol regulatory element binding protein-1c (SREBP-1c) pathways are key to the transcriptional control of hepatic fatty acid oxidation and lipogenesis, respectively (reviewed in (Ferre and Foufelle, 2007; Mandard et al., 2004)).

Retinoic acid (RA), the carboxylic acid and main active form of vitamin A, is an important regulator of mammalian adiposity and lipid metabolism, acting mostly at the gene expression level (Bonet et al., 2012). RA influences gene expression by an array of mechanisms, notably by binding to and activating two types of retinoid receptors: the retinoic acid receptors (RARs, α, β, and γ isoforms), which respond to both all-*trans* RA (ATRA) and 9-*cis* RA, and the retinoid X receptors (RXRs, α, β, and γ isoforms), which respond specifically to the 9-*cis* RA isomer (reviewed in (Bastien and Rochette-Egly, 2004)). Like the PPARs and the LXRs, the retinoid receptors belong to the nuclear receptor superfamily of ligand-modulated transcription factors. RAR:RXR heterodimers control the expression of typical retinoid-target genes. In addition, RXRs serve as the obligate heterodimer partners for other nuclear receptors. The transcriptional activity of some of these heterodimers – so-called permissive – responds to ligands of either partner, even in a synergistic manner when both ligands are bound, providing a mechanism for widespread effects of retinoids on gene expression (Aranda and Pascual, 2001). In particular, PPARs and LXRs involved in the control of liver lipid metabolism can act on target genes as permissive heterodimers with RXR (Willy et al., 1995; Mukherjee et al., 1997). Permissivity of RXR heterodimers might be gene-specific or dependent on cellular environment (Lalloyer et al., 2009; Pinaire and Reifel-Miller, 2007).

* Corresponding author at: Laboratory of Molecular Biology, Nutrition and Biotechnology, Universitat de les Illes Balears. Cra. Valldemossa, km 7.5, 07122 Palma de Mallorca, Spain. Tel.: +34 971 259506; fax: +34 971 173426.
E-mail address: joan.ribot@uib.es (J. Ribot).

1357-2725/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocel.2012.07.026

The action of retinoids on liver lipid metabolism is controversial. Studies in rodents fed high vitamin A doses (Ramachandran et al., 1975) or treated with ATRA (Amengual et al., 2010; Berry and Noy, 2009) pointed to an activity of retinoids favoring liver fatty acid catabolism and the reduction of hepatic lipid accumulation. This activity is also supported by findings of hepatosteatosis and decreased hepatic expression of mitochondrial fatty acid oxidation-related genes in vitamin A-deficient mice (Kang et al., 2007) and mouse models of genetic disruption of retinoid signaling (Yanagitani et al., 2004; Hessel et al., 2007). However, other studies have reported a shift toward increased fat catabolism in liver of rodents with vitamin A deficiency (McClintick et al., 2006; Oliveros et al., 2007), or increased hepatic lipogenesis and VLDL production following retinoid treatment in rodents and humans (Lalloyer et al., 2009; Gerber and Erdman, 1982). *In vivo* studies reflect pleiotropic systemic effects of retinoids which add to the intrinsic complexity of cellular retinoid signaling, making it difficult to ascertain direct effects of retinoids on hepatocyte metabolism. Previous studies in human hepatoma HepG2 cells have reported the induction of lipogenic gene expression (SREBP-1c and fatty acid synthase, FAS) by ATRA (Roder and Schweizer, 2007; Roder et al., 2007), but did not examine the retinoid impact on fatty acid catabolism. This scenario prompted us to study cell-autonomous effects of ATRA on lipid metabolism, and particularly fatty acid oxidation, in hepatic cells in culture. We present evidence that in HepG2 cells, a human hepatoma cell line widely used as a hepatocyte cell model, ATRA increases palmitate oxidation rate and up-regulates the expression of carnitine palmitoyl transferase-1 (CPT1-L), the rate-limiting enzyme in mitochondrial fatty acid oxidation through direct RXR-mediated mechanism(s).

2. Materials and methods

2.1. Chemicals

ATRA, 9-*cis* RA, actinomycin D, cycloheximide, cholecalciferol (vitamin D_3), isopropyl-(E,E)-(R,S)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (methoprene), and p-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid (TTNPB) were purchased from Sigma (St. Louis, MO). N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-benzene-sulfonamide (T0901317), and [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl] thio]-acetic acid (Wy14643) were from Cayman Chemicals (Ann Arbor, MI).

2.2. Cell culture and treatments

HepG2 cells, a human hepatoma-derived cell line, were obtained from ATCC (American Type Culture Collection; LGC Deselaers SL, Barcelona, Spain) and maintained in minimum essential medium (MEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Linus, Madrid, Spain), antibiotics (50 IU penicillin/mL and 50 mg streptomycin/mL) and 3 mM glutamine at 37 °C, in a humidified atmosphere with 5% CO_2. For all experiments, cells (4×10^4–6×10^4) were plated in 12-well cell culture plates and cultured for 4–5 days, with medium changes every 2–3 days, till they reached ~80% confluence. At this point, and after changing the medium, cultured cells were treated with the different drugs dissolved in DMSO, at the doses and for the times indicated in the text or in the figure and table legends. Control cells received the vehicle (DMSO). For experiments involving actinomycin D and cycloheximide, both chemicals were also dissolved in DMSO and added to the cells 4 h before ATRA treatment. DMSO final concentration never exceeded 0.1% in all treatments.

2.3. RNA isolation

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Isolated RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NadroDrop Technologies Inc., Wilmington, DE) and its integrity confirmed by agarose gel electrophoresis.

2.4. Real-time quantitative PCR (RT-qPCR) analyses

Real-time polymerase chain reaction was used to measure the mRNA expression levels of: CPT1-L (liver isoform), PPARα, RXRα, uncoupling protein 2 (UCP2), fatty acyl-CoA oxidase 1 (ACOX1), SREBP-1c, FAS, and β-actin, the latter as internal control. In brief, 0.25 μg of total RNA (in a final volume of 5 μL) was denatured at 65 °C for 10 min and then reverse transcribed to cDNA using MuLV reverse transcriptase (Applied Biosystems, Madrid, Spain) and random hexamers primers in an Applied Biosystems 2720 Thermal Cycler. Each PCR was performed from diluted (1/20) cDNA template, forward and reverse primers (1 μM each), and Power SYBR Green PCR Master Mix (Applied Biosystems, Madrid, Spain) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). The following primers were used: for CPT1-L, 5′-GATTTTGCTGTCGGTCTTGG-3′ and 5′-CTCTTGCTGCCTGAATGTGA-3′; for PPARα, 5′-TCATCAAGAAGACGGAGTCG-3′ and 5′-CGGTTACCTACAGCTCAGAC-3′; for RXRα, 5′-ACGAGAATGAGGTGGAGTCG-3′ and 5′-ATGTTGGTGACAGGGTCGTT-3′; for UCP2, 5′-GCCAGCCGACAGACACAG-3′ and 5′-CAGCAACAAGACGAGATAGAGGA-3′; for ACOX1, 5′-TGGTGAAGAAGATGAGGGAGT-3′ and 5′-AGCAAGGTGGGCAGGAAC-3′; for SREBP-1c, 5′-TGAGGACAGCAAGGCAAAG-3′ and 5′-CAGGACAGGCAGAGGAAGAC-3′; for FAS, 5′-GAGGAAGGAGGGTGTGTTTG-3′ and 5′-CGGGGATAGAGGTGCTGA-3′; and for β-actin, 5′-ACGGGCATTGTGATGGACTC-3′ and 5′-GTGGTGGTGAAGCTGTAGCC-3′. Primers were obtained from Sigma (Madrid, Spain). In order to verify the purity of the PCR products, a melting curve was produced after each run according to the manufacturer's instructions. The relative expression of each mRNA was calculated according to Pfaffl (2001), using β-actin as reference gene.

2.5. Immunoblotting analysis

Cultured cells were harvested in phosphate-buffered saline (PBS), immediately pelleted, resuspended in PBS supplemented with protease inhibitors, and then sonicated. The cell lysates were used for total protein quantification with the BCA protein assay kit (Pierce, Rockford, IL). Total protein (10 μg) was fractionated by SDS-polyacrylamide gel electrophoresis (8% polyacrylamide), and electrotransferred onto PVDF membranes (Bio-Rad, Madrid, Spain). Black amido B10 protein staining provided a visual evidence for correct loading and blotting. Membranes were blocked in a 5% non-fat powdered milk in T-PBS (phosphate-buffered saline, pH 7.5, containing 0.1% Tween 20), washed and incubated overnight at 4 °C with the primary antibody (polyclonal antibody to CPT1 raised in rabbits, from Santa Cruz Biotechnology, Santa Cruz, CA), according to the manufacturer's instructions. Horseradish peroxidase-conjugated anti-rabbit IgG antibody (Amersham Biosciences, Buckinghamshire, UK) was used as secondary antibody. The immunocomplexes were revealed using an enhanced chemiluminescence detection system (Amersham Biosciences). Membranes were exposed to Hyperfilm ECL (Amersham Biosciences), and bands in films were scanned using the Chemigenius BioImaging System (Syngene, Frederick, MD) and quantified using the GeneTools Software (Syngene).