FEBS Letters 580 (2006) 3361–3367

CYP4A11 is repressed by retinoic acid in human liver cells

Joseph Antoun[a], Yolande Amet[a], Brigitte Simon[a], Yvonne Dréano[a], Anne Corlu[b], Laurent Corcos[a,c], Jean Pierre Salaun[a,d], Emmanuelle Plée-Gautier[a,d,*]

[a] *EA-948 Laboratoire de Biochimie, Faculté de Médecine et des Sciences de la Santé, 22 Avenue Camille Desmoulins, CS 93837, 29238 Brest Cedex 3, France*
[b] *INSERM U-522, CHU Pontchaillou, 35033 Rennes Cedex, France*
[c] *INSERM U-613, CHU Morvan, Faculté de Médecine, Brest, France*
[d] *CNRS-UMR 7139 Station Biologique, 29682 Roscoff, Cedex, France*

Received 20 March 2006; revised 28 April 2006; accepted 3 May 2006

Available online 11 May 2006

Edited by Robert Barouki

Abstract **CYP4A11, the major fatty acid ω-hydroxylase in human liver is involved in the balance of lipids, but its role and regulation are both poorly understood. We studied the effects of retinoids on the regulation of CYP4A11 in the human hepatoma cell line HepaRG. Treatment of HepaRG cells with all-*trans*-retinoic acid resulted in a strong decrease in CYP4A11 gene expression and apoprotein content and, furthermore, was associated with a 50% decrease in the microsomal lauric acid hydroxylation activity. Such a strong suppression of CYP4A11 expression by retinoids could have a major impact on fatty acid metabolism in the liver.**

Keywords: Cytochrome P450; Retinoic acid; Human hepatoma cells; HepaRG; Fatty acid hydroxylase; Liver

1. Introduction

Among the human cytochromes P450 (CYP), the fatty acid ω-hydroxylase CYP4A sub-family is involved in the metabolism of medium- and long-chain fatty acids. The bioactivation of fatty acids to ω-hydroxymetabolites are believed to play an important role in the regulation of kidney function and systemic blood pressure [1]. CYP4A11 is the main fatty acid ω-hydroxylating enzyme expressed in human liver and kidney [2–4]. In the rat, the expression of CYP4A1, the major fatty acid hydroxylase of the liver, is regulated by physiological conditions including diabetes and fasting [5–7] and can be induced by different compounds, e.g., fatty acids and peroxisome proliferators like hypolipidemic drugs [8]. These effects are considered to be mediated via the peroxisome proliferator-activated receptor alpha (PPARα) [6].

Because of the lack of appropriate cell systems, the regulation of human CYP4A gene expression is poorly documented. In HepG2 hepatoma cells in which was overexpressed a murine PPARα mutant exhibiting a significantly decreased ligand-independent *trans*-activation potential, CYP4A11 is induced by peroxisome proliferators [9]. In primary cultures of human hepatocytes, the PPARα agonist, clofibrate, increases CYP4A11 mRNA [10].

CYP4A members exhibit a marked physiological role in the maintenance of lipid homeostasis in rodents, especially as PPARα responsive genes, but the existence of such a mechanism in humans is still debated.

The lipid-soluble vitamin A and its derivatives, all-*trans*-retinoic acid (ATRA) and 9-*cis*-retinoic acid (9-*cis*-RA), exert various effects on lipid metabolism by interrupting lipid peroxidation chain reaction (neutralising singlet oxygen) and regulating enzymes involved in the metabolism of triglycerides [11,12], cholesterol [12,13] and fatty acids [14,15]. In rats, supplementation with β-carotene induces several CYP genes including CYP1A1/2, CYP3A1/2, CYP2E1, CYP2B1/2 and CYP2C11 in the liver [16].

Retinoids are natural and synthetic derivatives of retinol, and among them ATRA and its stereoisomer, 9-*cis*-RA, are biologically active. By their ability to modulate cell proliferation, differentiation and apoptosis, depending on the cell phenotype, they both control carcinogenesis. Retinoids act as chemopreventive agents in rats over the initial phase of hepatocarcinogenesis [17,18], but induce hepatic cell proliferation in normal mice [19]. In the human hepatoma cell line HepG2, retinoic acid induces growth arrest and differentiation [20]. The survival rate of patients with hepatocarcinoma was significantly prolonged by a 12-months postoperative treatment with retinoids as adjuvant treatment [21].

Retinoids exert their effects activities through interactions with the retinoic acid receptor (RAR) and retinoid X receptor (RXR) nuclear receptors found as three isotypes (α, β and γ). These ligand-activated receptors act as transcription factors and lead to transcriptional stimulation or repression by binding to RA response elements in the control region of numerous target genes. Moreover, both are essential in the regulation of gene transcription by retinoids. RXR also plays a unique role in the nuclear receptor family as a common heterodimeric partner for several other nuclear receptors during transcription

*Corresponding author. Fax: +33 2 98 01 66 03.
E-mail address: emmanuelle.plee-gautier@univ-brest.fr (E. Plée-Gautier).

Abbreviations: ATRA, all-*trans*-retinoic acid; Bezaf, bezafibrate; 9-*cis*-RA, 9-*cis*-retinoic acid; CYP, cytochrome P450; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ω-LAH, lauric acid ω-hydroxylase; LTB4, leukotriene B4; PGJ2, 15-deoxyprostaglandine J2; PPAR, peroxisome proliferator-activated receptor; 2BrP, 2-bromopalmitate; RAR, retinoic acid receptor; RARE, retinoic acid response element; RXR, retinoid X receptor

0014-5793/$32.00
doi:10.1016/j.febslet.2006.05.006

modulation. The investigations reported here were aimed at assessing whether retinoids can regulate the CYP4A11 fatty acid hydroxylase in human hepatoma cells. The HepaRG cell line being the first human hepatoma cell line to stably express high levels of the main CYPs involved in xenobiotic metabolism [22], it appeared to be a relevant in vitro model; it also expresses genes implicated in lipid metabolism, including PPARα, SREBP1c, fatty acid synthase (FAS), CYP3A4 and CYP4F3 at levels close to those found in liver biopsies [23] (and unpublished data). In the present study, we provide evidence for a strong downregulation by ATRA of CYP4A11 gene expression, protein and associated catalytic activities.

2. Materials and methods

2.1. Chemicals

Retinoic acids, L165,041, bezafibrate (Bezaf) were from Sigma (Saint Quentin Fallavier, France). Lauric acid was obtained from Fluka (Buchs, Switzerland). Radiolabeled [1-^{14}C]-lauric acid (1.85 GBq/mmol) was from Amersham Biotech (Amersham, UK). Culture media and glutamine were purchased from Gibco BRL (Cergy-Pontoise, France), sera from Perbio (France), insulin from Sigma and hydrocortisone hemisuccinate from Upjohn Pharmacia (Guyancourt, France). Electrophoresis reagents were from Bio-Rad (France), nitrocellulose sheets from Amersham Biotech and immunoblot primary antibodies from RDI (Research Diagnostics Incorporation, Flanders, NJ). Modified cell microsomes containing human P450s were obtained from Gentest (Woburn, MA). All other chemicals and solvents were of analytical grade from Sigma or VWR International (Pessac, Fance).

2.2. Cell culture

Hepa RG cells were seeded in six-well plates in 2 mL of complete medium composed of William's E media supplemented with 5 U/mL penicillin, 5 μg/mL streptomycin, 10% fetal calf serum (HYCLONE: Fetalclone III Bovine serum product), 200 mM L-glutamine, 5 μg/mL insulin and 5×10^{-5} M hydrocortisone. After 12 days, the medium was supplemented at first with 1% DMSO and then with 2% DMSO till the 24th day, when the different treatments were applied.

2.3. RNA extraction and detection of RNA damage

Total RNA was extracted using the ABI Prism™ 6100 Nucleic Acid PrepStation (Applied Biosystems, France). The cells were washed with PBS, and the samples were processed on the 'Nucleic Acid PrepStation' using the 'Applied Biosystems Total RNA Chemistry' according to the manufacturer's instructions. The purified RNA was eluted in a total volume of 150 μL. The integrity of total RNA was checked on an Agilent 2100 Bioanalyser using the RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA).

2.4. Reverse transcription and TaqMan polymerase chain reaction

Two hundred nanograms of total RNA (10 μL) were reverse-transcribed into cDNA using the high-capacity cDNA Archive Kit with random hexamers according to the supplier's instructions. The reporter for the TaqMan probes, all purchased from Applied Biosystems, was FAM. A TaqMan Universal PCR Master Mix was mixed with the designed assays as follows: 12.5 μL of 2× Master mix, 0.62 μL of the cDNA solution and 1.875 μL of water. The temperature program was 50 °C for 2 min, 95 °C for 10 min, 95 °C for 9 s and then 60 °C for 1 min for 40 cycles. Data were normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal.

2.5. Microsome preparation

Once the cells had been washed with PBS, each dish was scratched in 1 mL of 50 mM phosphate buffer, pH 7.4, containing 0.25 M saccharose, 10 mM EDTA and 0.1 mM dithiothreitol (DTT). The cells were grinded in a glass potter in ice and sonicated for 10 s. Then, the mix was centrifuged at $10000 \times g$ for 20 min at 4 °C; the supernatant was collected and centrifuged at $105000 \times g$ for 60 min at 4 °C. The pellet was washed in 0.5 mL of 100 mM phosphate buffer, pH 7.4, containing 10 mM EDTA and 0.1 mM DTT. The microsomal subcellular fraction was stored until use at −80 °C in 0.4 mL of 100 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, 20 mM DTT and 20% glycerol (v/v). Microsomal protein content was determined using the Bio-Rad protein assay based on the Bradford dye-binding procedure with bovine serum albumin as standard.

2.6. Assay of monooxygenase enzymatic activities

The ω and (ω − 1)-hydroxylations of lauric acid were determined by HPLC as previously reported in microsomes from rat- and human-liver [24,25] using lauric acid (0.1 mM; specific activity 74 kBq/mL) as substrate.

2.7. RP-HPLC analysis

The ω and (ω − 1)-hydroxylated metabolites of lauric acid and residual substrate were separated by RP-HPLC using a 5-μm Ultrasphere C18 column 150 × 4.6 mm (Beckman, France) as previously described [24,28]. The HPLC chromatography apparatus was equipped with a Flo-One Beta radiometric detector (Packard, Meriden, CT). Metabolic rates were calculated from the percent-transformed areas of metabolites and expressed in pmol min^{-1} mg^{-1} of protein.

2.8. Immunoquantification of CYP4A11 protein

Aliquots of cell microsomal preparations (100 μg) were separated by electrophoresis on 9% SDS–polyacrylamide gels and then electrophoretically transferred to nitrocellulose sheets. These sheets were blocked with TBS containing 5% (w/v) BSA and 0.1% (v/v) Tween 20 for 1 h at room temperature before overnight incubation, at 4 °C, with rabbit anti-CYP4A11 primary polyclonal antibody. After washing with TBS/tween, they were treated with TBS containing anti-rabbit Ig-biotinylated species-specific secondary antibody conjugated to streptavidine-horseradish peroxidase and then washed with TBS/tween. The peroxidase activities were evidenced by ECL detection using luminol. Quantification of the blots was performed by image processing scan analysis (Gel Doc XR, Biorad, France); the integrated peaks were expressed as arbitrary units relative to protein quantity.

Relative amounts of protein loaded and transferred onto the blot were assessed by Coomassie blue staining and quantified by image processing scan analysis (Gel Doc XR); the coloration of each lane varied no more than 5%.

2.9. Statistical analysis

The results are expressed as means ± S.D. The differences between control and treatment groups were assessed by analysis of variance followed with Student's t tests and considered as statistically significant when P was <0.05.

3. Results

3.1. Dose- and time-dependent decrease of CYP4A11 mRNA level by ATRA

In order to study the effect of ATRA on CYP4A11 gene regulation, fully differentiated HepaRG cells were incubated with increasing concentrations of ATRA in the medium. Unexpectedly, CYP4A11 mRNA was quickly downregulated by retinoids (Fig. 1A); its half life was about 5 and 7 h for 1 and 10 μM ATRA, respectively. An 80% reduction was observed for both doses after 12 h. The EC_{50} was 5 nM after 18 h (Fig. 1B). These effective ATRA concentrations are similar to those reported for human plasma retinal (1.7–2 μM) [26] and ATRA (8–16 nM) [27]. A strong downregulation of CYP4A11 expression was also obtained in HepG2 human hepatoma cells 18 h after the treatment by 1 or 10 μM ATRA (51% and 69% inhibition, respectively).

3.2. Reduction of CYP4A11 protein content by ATRA

To determine whether the decrease in CYP4A11 mRNA level was associated with a reduction of CYP4A11 protein