Biochemical Pharmacology 84 (2012) 701–711

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

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

DEC1 binding to the proximal promoter of CYP3A4 ascribes to the downregulation of CYP3A4 expression by IL-6 in primary human hepatocytes

Zhao Mao [a,c,1], Xiaofei Luan [a,1], Gang Cao [c], Wei Liu [a], Jing Xiong [a], Gang Hu [a], Ruini Chen [a], Rui Ning [a], Wei Shang [a], Jian Yang [a,*], Bingfang Yan [b,**]

[a] Department of Pharmacology, Nanjing Medical University, Nanjing, Jiangsu 210029, China
[b] Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, 02881, USA
[c] Nanjing Jinling Hospital, 210002, China

ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 13 June 2012
Accepted 13 June 2012
Available online 21 June 2012

Keywords:
Cytochrome P450 3A4 (CYP3A4)
Interleukin 6 (IL-6)
Differentially expressed in chondrocytes 1 (DEC1)
Transcription regulator

ABSTRACT

In this study, we provided molecular evidences that interleukin-6 (IL-6) contributed to the decreased capacity of oxidative biotransformation in human liver by suppressing the expression of cytochrome P450 3A4 (CYP3A4). After human hepatocytes were treated with IL-6, differentially expressed in chondrocytes 1 (DEC1) expression rapidly increased, and subsequently, the CYP3A4 expression decreased continuously. Furthermore, the repression of CYP3A4 by IL-6 occurred after the increase of DEC1 in primary human hepatocytes. In HepG2 cells, knockdown of DEC1 increased the CYP3A4 expression and its enzymatic activity. In addition, it partially abolished the decreased CYP3A4 expression as well as its enzymatic activity induced by IL-6. Consistent with this, overexpression of DEC1 markedly reduced the CYP3A4 promoter activity and the CYP3A4 expression as well as its enzymatic activity. Using sequential truncation and site directed mutagenesis of CYP3A4 proximal promoter with DEC1 construct, we showed that DEC1 specifically bound to CCCTGC sequence in the proximal promoter of CYP3A4, which was validated by EMSA and ChIP assay. These findings suggest that the repression of CYP3A4 by IL-6 is achieved through increasing the DEC1 expression in human hepatocytes, the increased DEC1 binds to the CCCTGC sequence in the promoter of CYP3A4 to form CCCTGC–DEC1 complex, and the complex downregulates the CYP3A4 expression and its enzymatic activity.

1. Introduction

The liver is the richest source of drug metabolism [1], which is regulated by the expression of drug-metabolism enzymes [1,2]. Cytochrome P450 3A4 (CYP3A4) is the most important human CYP in the liver and small intestine and plays a major role in the biotransformation of many drugs. Actually, it is responsible for the oxidative metabolism of more than 60% of all pharmaceuticals [3,4] and its activity shows a wide inter-individual variability, which forms a basis for clinically significant drug interactions and toxicities [5,6]. Cytokines such as tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6), which increase in inflammatory diseases and some cancers [7,8], have been shown to down-regulate the expression of a variety of drug-metabolizing enzymes including CYP3A4 [7,9–11]. Transactivation by nuclear receptors such as pregnane X receptor (PXR) and constitutive androstane receptor (CAR) is largely responsible for the increased expression of these genes [2]. Some studies have reported that the decrease of CYP3A4 enzyme expression is associated with the repression of CAR and PXR in mouse liver during the acute phase response [12] and in human hepatocytes mediated by IL-6 [11]. However, other mechanisms may also contribute to the down-regulation of CYP3A4 in inflammation, because CYP3A4 decreases to a larger extent compared to PXR in response to cytokines such as IL-6 [11].

Human DEC (differentially expressed in chondrocytes, DEC), mouse STRA13 (stimulated with retinoic acid 13), and rat SHARP (split and hairy related protein) constitute a new and structurally distinct class of basic helix-loop-hellix (bHLH) proteins [13–15]. The bHLH proteins are intimately associated with the developmental events such as cell differentiation and lineage commitment

Abbreviations: DEC1, differentially expressed in chondrocytes 1; IL-6, interleukin-6; CYP450, cytochrome P450; PXR, pregnane X receptor; hPXR, human pregnane X receptor; Rif, rifampicin; TNF-α, tumor necrosis factor α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; bHLH, basic helix-loop-hellix; HLH, helix-loop-hellix; SHARP, split and hairy related protein; STRA13, stimulated with retinoic acid 13; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; EMSA, electrophoretic mobility shift assay; ChIP, chromatin immunoprecipitation.

* Corresponding author. Tel.: +86 25 86863159; fax: +86 25 86863159.
** Corresponding author.
E-mail addresses: jianyang@njmu.edu.cn, clinic_pharm1208@yahoo.com (J. Yang).

[1] The authors contributed equally to this work.

0006-2952/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2012.06.010

[16–18]. The HLH domain in the bHLH motif is responsible for dimerization, whereas the basic region mediates DNA binding [19]. Two members of DEC/STRA/SHARP proteins are identified in each mammalian species studied with a sequence identity of >90% in the bHLH region and ~40% in the total proteins, respectively [14]. They are called DEC1 and DEC2, exhibiting an overlapping tissue distribution. DEC/STRA/SHARPs have been implicated in cell differentiation [14,20], maturation of lymphocytes [21], regulation of molecular clock [22], and involvement in maintaining the homeostasis of metabolism [23]. In addition, DEC1 expression is highly elevated in response to environmental stimuli such as hypoxia and cytokines such as TNFα [24,25].

In this study, we report that DEC1 is a transcriptional repressor of CYP3A4, a major human enzyme that plays an important role in oxidative biotransformation [26]. After human hepatocytes were treated with IL-6, the DEC1 expression rapidly increased, then, the expression of CYP3A4 decreased at both mRNA and protein levels. Meanwhile, the repression of CYP3A4 by IL-6 occurred after the increase of DEC1 in primary human hepatocytes. So we hypothesize DEC1 is a transcriptional repressor of CYP3A4, and DEC1 induction is one of important mechanisms of the reduction of CYP3A4 expression by IL-6 in primary human hepatocytes.

2. Materials and methods

2.1. Chemicals and supplied

IL-6 was purchased from R & D Systems (Minneapolis, MN, USA). FlagCMV2 vector, rifampicin and Williams'E medium were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium, high-fidelity platinum Taq DNA polymerase, and insulin-transferrin-selenium G supplement were purchased from Invitrogen (Carlsbad, CA, USA). GeneJet™ DNA VitroTransfection Reagent (Ver II) was from SignaGen Labortories (Gaithersburg, MD, USA). Dual-luciferase reporter assay system and P450-Glo™ Luminescent cytochrome P450 3A4 Assay system were purchased from Promega (Madison, WI, USA). Fetal bovine serum was from Hyclone Laboratories (Logan, UT, USA). The antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Abcam (Cambridge, UK). The goat anti-rabbit IgG conjugated with horseradish peroxidase from Pierce Chemical (Pierce, Rockford, IL, USA). Polyclone antibody (from rabbit) against DEC1 and CYP3A4 were kindly provided by Dr. Yan Lab (University of Rhode Island). Nitrocellulose membrane was from Bio-Rad Laboratories (Hercules, CA, USA). DEC1 shRNA plasmid and IgG were from Santa Cruz (Santa Cruz, CA, USA). Nuclear extraction kit, Chromatin Immunoprecipitation Assay Kit was from Active Motif, Inc (Carlsbad, CA, USA). [γ-^{32}P]ATP was from FuRui Bioengineering company (Beijing, China). All other reagents were from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Culture and treatment of human primary hepatocytes and hepatoma cells

Human primary hepatocytes in 6-well plates were obtained from the Liver Tissues Procurement and Distribution System (University of Minnesota, Minneapolis, MN or CellzDirect, Pittsboro, NC, USA). The ten hepatocyte donors were all non-smokers of four males (21–65 years old) and six females (35–72 years old) with seven white and three black. Upon the arrival of the hepatocytes, the culture media were replaced with Willians'E medium containing insulin-transferrin-selenium supplement and penicillin/streptomycin [27]. After incubation at 37 °C with 5% CO_2 for 24 h, the hepatocytes were treated with 10 ng/ml IL-6 for 24 h (for mRNA level) or 48 h (for protein level) [27]. Hepatoma (HepG2) cells were purchased from American Type Culture Collection (Mannassas, VA, USA), and maintained in the Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, penicillin/streptomycin, and 1 × nonessential amino acids. HepG2 cells were seeded at the density of 10^6 cells/well (6-well plates), 2.5×10^5 cells/well (12-well plates) and that of 8×10^4 cells/well (48-well plates) in a regular medium, and the treated cells were cultured in a 1% serum-reduced medium.

2.3. Quantitative reverse transcription-polymerase chain reaction

The total RNA was isolated by using a RNA-Bee (Tel-Test Inc., Friendswood, TX, USA) according to the manufacturer's instruction and checked by formaldehyde gel electrophoresis for quality control. The first-strand cDNA was synthesized using total RNA (1 μg) at 25 °C for 10 min, 42 °C for 50 min, and 70 °C for 10 min by using random primers and moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). The cDNAs were then diluted eight times and the quantitative PCR was conducted with TaqMan Gene Expression Assay kits (Applied Biosystems, Foster City, CA, USA). The TaqMan assay identification numbers are: CYP3A4, Hs00604506_m1; DEC1, Hs00186419_m1; GAPDH, 4352934E. A 20 μl PCR mix contained 10 μl of universal PCR master mixture, 1 μl of gene-specific TaqMan assay mixture (probe), 6 μl of diluted cDNA as template and 3 μl of water. The PCR amplification and quantification were done in an Applied Biosystems 7900 real-time PCR system (Applied Biosystems, Foster City, CA, USA) with one cycle at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The signals from each target gene were normalized based on the signal from GAPDH.

2.4. Plasmid constructs and site-directed mutagenesis

A cDNA encoding the full-length DEC1 was isolated by a cDNA-trapping method [19]. DEC1 mutant constructs were prepared by PCR with full-length DEC1 as the template as previously described [19]. CYP3A4-DP and CYP3A4P promoter reporters (−7836/−6093 to −362/ + 53, −362/ + 53) were prepared as previously described [27,28]. A set of deleted and mutated constructs targeted on proximal promoter, which were presented in Fig. 4A, were prepared by PCR using the targeting primers shown in Tables 1 and 2. The fragments harboring these elements were amplified by PCR with primers that were extended to include appropriate endonucleases (XhoI/HindIII) to facilitate the subsequent ligation. The sequences of all of the CYP3A4 reporter gene constructs were verified by direct DNA sequencing.

2.5. Transient co-transfection experiment

HepG2 cells were plated in 48-well plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at the density of 8×10^4 cells/well. Transfection was conducted by GeneJet™ DNA VitroTransfection Reagent (Ver II). The transfection mixtures contained 50 ng of a reporter plasmid (CYP3A4-DP-luc), 50 ng of hPXR plasmid, a corresponding concentration of FlagDEC1 along with 5 ng of *Null-Renilla reniformis* luciferase plasmid. Vector plasmid was used to equalize the amount of plasmid DNA for each transfection. HepG2 cells were transfected for 24 h and the transfected cells were treated with rifampicin (Rif) 10 μM for another 24 h. The cells were washed once with phosphate-buffer saline (PBS) and then lysed by 1 × passive lysis buffer (Promega, Madison, WI). The collected cells were subjected to two cycles of freeze/thaw. The reporter enzyme activities were assayed with Dual-Luciferase reporter assay system. This system contained two substrates, the firefly luminescence and *Renilla* luminescence, which were used to