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Short communication

Induction of human cholesterol 7α-hydroxylase in HepG2 cells by 2,4,6-trihydroxyacetophenone

Juree Charoenteeraboon[a], Kasem Nithipatikom[b], William B. Campbell[b], Pawinee Piyachaturawat[c], Prapon Wilairat[a], Pornpimol Rongnoparut[a,*]

[a]Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Rd, Phyatai, Bangkok 10400, Thailand
[b]Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA
[c]Department of Physiology, Faculty of Science, Mahidol University, Rama VI Rd, Phyatai, Bangkok 10400, Thailand

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Abstract

In animal the plasma cholesterol-lowering activity of 2,4,6-trihydroxyacetophenone (THA) is due to enhanced cholesterol 7α-hydroxylase (CYP7A1) activity. We have examined the effect of THA on CYP7A1 activity and mRNA level in HepG2 cells. THA stimulated CYP7A1 activity in a concentration- and time-dependent manner. After exposure for 24 h, 1 μM THA induced CYP7A1 activity 160±8% and mRNA level 166±21% (mean±S.E.M.) of control. Moreover THA antagonized the inhibitory regulation of chenodeoxycholic acid on CYP7A1 mRNA expression. These results indicated that THA increases CYP7A1 activity in human HepG2 cells by stimulating mRNA transcription.

Keywords: Chenodeoxycholic acid; Cholesterol 7α-hydroxylase; CYP7A1; HepG2 cell; Induction; 2,4,6-Trihydroxyacetophenone

1. Introduction

Cholesterol 7α-hydroxylase (CYP7A1) is the first and rate-limiting enzyme in bile acid synthesis pathway and is expressed only in the liver (Myant and Mitropoulos, 1977). Lack of CYP7A1 results in high levels of plasma cholesterol (Pullinger et al., 2002), whereas induction of CYP7A1 prevents elevation of blood cholesterol in rodents fed a cholesterol-rich diet (Dueland et al., 1993) indicating its importance in maintaining plasma cholesterol homeostasis. CYP7A1 is tightly regulated by feed-forward of cholesterol and negative feedback of bile acids. Bile acids indirectly repress the transcription of *CYP7A1* through the farnesoid X receptor (FXR). However, regulation of *CYP7A1* by oxysterols derived from cholesterol differs among various species. In human *CYP7A1* cannot respond to cholesterol load as in rodents due to the lack of the liver X receptor α (LXRα) responsive element in the promoter of human *CYP7A1* (Chiang et al., 2001; Gupta et al., 2002).

Recently, 2,4,6-trihydroxyacetophenone (THA), the aglycone part of acetophenone glycoside obtained from *Curcuma comosa* Roxb., has been reported to have a cholesterol-lowering activity in animal as a result of enhanced bile acid and cholesterol excretion by an induction of CYP7A1 activity (Piyachaturawat et al., 2000, 2002a). THA was the most effective compound among the hydroxy analogs (Piyachaturawat et al., 2000) and has low toxicity in animal (Piyachaturawat et al., 2002b), but its role in man has not been investigated. This study examined the effect of THA on human CYP7A1 activity and mRNA level in human hepatocarcinoma HepG2 cells and explored the mechanism of CYP7A1 induction using chenodeoxycholic acid (CDCA). The results demonstrated that THA increased both CYP7A1 enzyme activity and mRNA level in human HepG2 cells and could antagonize the inhibitory effect of CDCA on CYP7A1 mRNA expression.

* Corresponding author. Tel.: +66 2 201 5453; fax: +66 2 354 7174.
E-mail address: scprn@mahidol.ac.th (P. Rongnoparut).

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2. Materials and methods

HepG2 cells, hepatocarcinomas, were obtained from the ATCC (Manassas, VA, USA). Cells were maintained in Modified Eagle's medium (MEM) alpha (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Hyclone, Longan, UT, USA) and 1% glutamine and incubated at 37 °C in a 5% CO_2 atmosphere. Before treatment, cells were starved in serum-free medium for 24 h. HepG2 cells were treated with 0.1–25 μM THA (Sigma-Aldrich, MO, USA) alone or in combination with 25 μM CDCA (Sigma-Aldrich) before harvesting. Ethanol was used as control vehicle.

To assay for CYP7A1 enzyme activity, microsomes were isolated from HepG2 cells using differential fractionation (Morimoto and Sabatini, 1998). The microsomal pellet was suspended in 200 μl of 0.1 M phosphate buffer, pH 7.5, containing 0.1 mM EDTA, 5 mM dithiotherol, 5 mM $MgCl_2$, 50 mM NaF and 0.25 mM sucrose using a hand homogenizer. CYP7A1 assays were carried out in 500 μl containing 0.5 mg microsomal protein, 50 μM cholesterol, 50 μM oleandomycin, and NADPH regenerating system (1 mM NADPH, 5 mM isocitric acid, 0.01 U of isocitrate dehydrogenase). A 5 min preincubation was performed at 37 °C prior to addition of microsomal protein. The reaction was stopped by ethanol. β-Sitosterol (Steroloids, Wilton, NH, USA) was added to the sample as an internal standard. The product was extracted using Bond Elut C18 cartridge (Varian, Harbor City, CA) and further derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetaminde (Supelco, PA, USA). The 7α-hydroxycholesterol was analyzed by GC-MS (a Hewlett-Packard 5890 Series II Gas Chromatograph-5989A Engine Mass Spectrometer) with selected-ion monitoring. The 7α-hydroxycholesterol and β-sitosterol were monitored at *m/z* 456 and 396, respectively. Protein concentration was determined by a protein assay kit (Bio-Rad, Hercules, CA, USA).

Quantification of gene expression by real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed in iCycler detection system using SYBR Green Mastermix (Bio-Rad). Total RNA was isolated from cells using TRIzol reagent according to the manufacturer's instruction (Invitrogen, CA, USA) and reverse-transcribed to cDNA using cDNA Synthesis Kit (Bio-Rad). PCR amplifications were conducted using one denaturation step at 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s, 58 °C for 1 min, and 1 cycle of 95 °C for 1 min and 55 °C for 1 min. Pimers specific for human CYP7A1 cDNA (CYP7A1(+), 5′CACTTTGTCCACCTTTGATG3′; CYP7A1(−), 5′GCTGCTTTCATTGCTTCTG3′) and for human GAPDH cDNA (GAPDH (+), 5′GAAGGTGAAGGTCGGAGTC3′; GAPDH (−) 5′GAAGATGGTGATGGGATTTC3′, Cheung and Cheung, 2001) were used. Transcript levels of CYP7A1 mRNA were normalized to GAPDH mRNA levels.

Statistical analysis was performed using Student's *t*-test with a significance level of $P<0.05$ and <0.01.

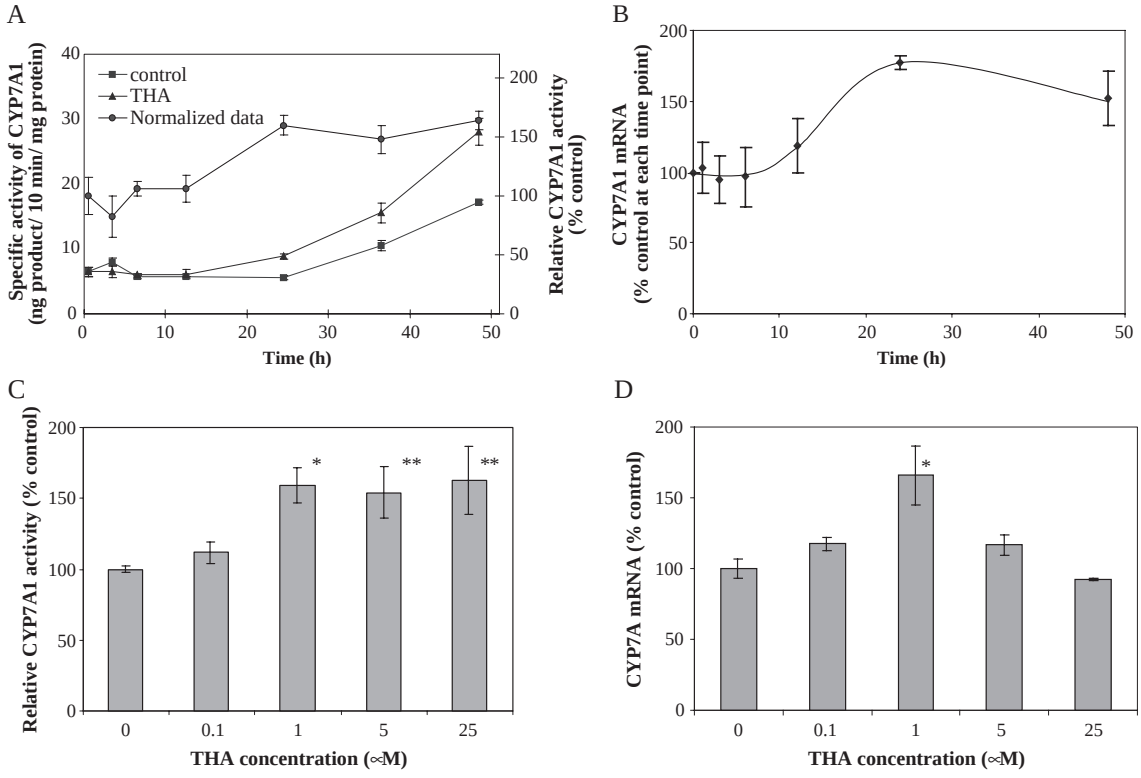

Fig. 1. THA treatment induces CYP7A1 activity and mRNA levels in Hep G2 cells. (A) Time course of CYP7A1 activity, expressed as specific activity (ng of 7α-hydroxycholesterol/10 min incubation/mg protein), in control HepG2 cells (■), 1 μM THA-treated HepG2 cells (▲), and relative CYP7A1 activity (paired time-point) in THA-treated HepG2 cells as compared with control (●). (B) Time course of CYP7A1 mRNA levels, expressed as % of untreatment (paired time-point), in response to 1 μM THA treatment of HepG2 cells. Effect of THA concentration (at 24 h incubation) on CYP7A1 activity (C) and mRNA (D) in HepG2 cells. Relative activity and mRNA level are expressed as percent of control. All values are mean±S.E.M.; $n=3–6$; *$P<0.01$ and **$P<0.05$.

3. Results

Microsomal CYP7A1 activity in untreated HepG2 cells increased during the incubation period of 48 h, and 1 μM THA stimulated the activity after 24 h of exposure (Fig. 1A). CYP7A1 enzyme activity of THA-treated cells at 24 h was 160±8.1% (mean±S.E.M.) of control. Stimulation on CYP7A1 enzyme activity by THA (at 24 h) was concentration-dependent over the range of 0–1 μM, and remained constant at the higher concentrations (Fig. 1C). The increase of CYP7A1 mRNA level, measured by using real-time RT-PCR to quantify CYP7A1 mRNA level, was similar to those obtained for enzyme activity (compare Fig. 1A and B, C and D). These results indicated that THA stimulated CYP7A1 activity by elevating mRNA level, although there was a decline in stimulation at the high concentrations.

Previous studies (Li et al., 1990) have revealed that CYP7A1 expression is negatively regulated at the transcriptional level by its bile acid products. Accordingly, we examined whether THA could oppose the suppression of CYP7A1 expression by CDCA, which, among the bile acids, has the strongest inhibitory effect (Ellis et al., 2003). HepG2 cells were treated for 24 h with CDCA (25 μM) alone or in the combination with varying THA concentrations (0.1–25 μM). CYP7A1 mRNA levels of the CDCA-treated cells declined to 29% of the untreated cells (Fig. 2). Addition of THA at low concentrations (0.1–1 μM) resulted in an increase in CYP7A1 mRNA level of up to 60% of 25 μM CDCA-treated control cells (Fig. 2). However, this ability of THA to overcome the inhibitory effect of 25 μM CDCA on CYP7A1 mRNA level was not seen at the higher THA concentrations (5–25 μM).

4. Discussion

Conversion of cholesterol into bile acids is the only route for the disposal of cholesterol from the body. Human HepG2 cells that express CYP7A1 were employed to test the effect of THA that has previously been shown to be capable of stimulating CYP7A1 activity in animal (Piyachaturawat et al., 2002a). In this study, THA induced both CYP7A1 activity and mRNA level in HepG2 cells in a time- and concentration-dependent manner. The stimulatory effect of THA (1 μM) in HepG2 cells was similar to that seen for dexamethasone, which upregulates CYP7A1 mRNA level by 39% at an equivalent concentration (Taniguchi et al., 1994). A possible explanation for the low induction level is the limited amount of nuclear factors in HepG2 cells (Chiang et al., 2001; del Castillo-Olivares and Gil, 2000) that are required for CYP7A1 induction. At high concentrations of THA (5–25 μM), CYP7A1 mRNA levels declined, whereas enzyme activity levels remained constant (Fig. 1C and D). This could reflect the difference between the turnover rate of mRNA and enzyme; in rat, CYP7A1 mRNA half-life is 0.5 h (Baker et al., 2000) whereas that of protein is 2–4 h (Myant and Mitropoulos, 1977).

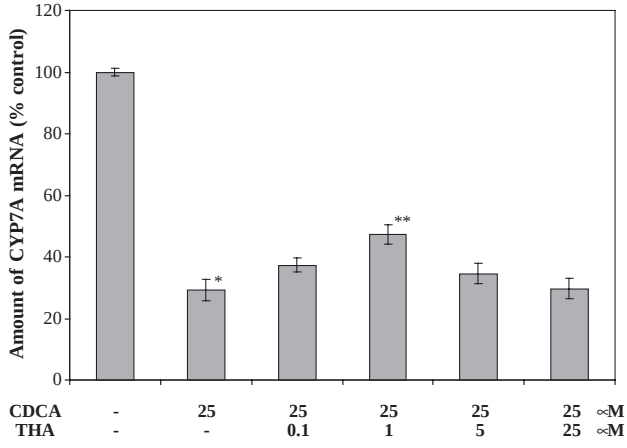

Fig. 2. THA antagonizes the reduction of CYP7A1 mRNA level by CDCA. After treatment with 25 μM of CDCA alone or in combination with THA (0.1–25 μM), the relative CYP7A1 mRNA levels are expressed as % of untreated HepG2 cells. Data are mean±range of two experiments, and $P<0.01$ when compared to untreated HepG2 cells (*) and to CDCA-treated HepG2 cells (**).

Elevation of CYP7A1 mRNA level can be due to an enhancement of transcription and/or an increase in mRNA stability. In the presence of actinomycin D, an RNA polymerase inhibitor, THA had no effect on CYP7A1 mRNA degradation rate (data not shown). FXR is the important regulator in human primary bile acid synthesis, since the transcriptional control of human *CYP7A1* does not involve the LXRα pathway (Chiang et al., 2001; Gupta et al., 2002). CDCA, the natural ligand of FXR, turns off the *CYP7A1* promoter by the activation of the small heterodimer protein (SHP-1) (Lu et al., 2000). THA stimulates the human *CYP7A1* at transcriptional level, as demonstrated by the ability of THA to reverse the depression of HepG2 CYP7A1 mRNA content by CDCA, which binds FXR and subsequently stimulates SHP-1 mRNA expression.

In summary, THA was shown to induce CYP7A1 activity in human HepG2 cells by increasing mRNA level. Increased enzyme activity paralleled mRNA level. THA or its analogues might constitute a new class of compounds that could be developed as possible candidates for future treatment of hypercholesterolemia.

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