การควบคุมการแสดงออกของจีนสร้างเอนไซม์ fructose-1,6-bisphosphatase ด้วย cAMP, dexamethasone และ PPAR-α ในเซลล์ตับ HepG2

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บทคัดย่อ

ฟรุคโตสบิสฟอสฟาเตส (fructose-1,6-bisphosphatase: FBP1) เร่งปฏิกิริยาการเปลี่ยน fructose-1,6-bisphosphate ไปเป็น fructose-6-phosphate ซึ่งเป็นตัวกลางในปฏิกิริยาการสร้างน้ำตาลกลูโคสด้วยวิธี gluconeogenesis ในตับ ความผิดปกติในการแสดงออกของ FBP1 เป็นสาเหตุให้เกิดโรคเบาหวานในสัตว์ทดลองรวมทั้งมนุษย์ คณะผู้วิจัยได้ทำการศึกษาฤทธิ์ของลิแกนด์ชนิดต่างๆ ได้แก่ forskolin, dexamethasone, all-trans retinoic acid และ PPAR-α agonist, WY-14643 ต่อระดับการแสดงออกของ FBP1 mRNA ในเซลล์ HepG2 โดยพบว่า forskolin, dexamethasone หรือ retinoic acid อย่างเดียวไม่มีผลต่อการเพิ่มระดับของ FBP1 mRNA ขณะที่เซลล์ HepG2 ที่ให้สาร WY-14643 ที่ความเข้มข้น 0.2 mM เป็นเวลา 48 ชั่วโมง และ 72 ชั่วโมง มีการเพิ่มระดับ FBP1 mRNA ร่วมกับจีน phosphoenolpyuvate carboxykinase-C และ glucose-6-phosphatase ยิ่งไปกว่านั้นการให้ WY-14643 ร่วมกับ forskolin ส่งผลให้มีการเพิ่มระดับ FBP1 mRNA ขึ้นไปอีก และเมื่อทำการวิเคราะห์ลำดับนิวคลีโอไทด์บริเวณโปรโมเตอร์จีน FBP1 ของมนุษย์ได้พบลำดับนิวคลีโอไทด์เป็นบริเวณที่จับของตัวรับ PPAR-α (PPRE) ที่ตำแหน่ง -358/-346 and -212/-198 ซึ่งส่งผ่านการออกฤทธิ์ของ WY-14643 ต่อการเพิ่มปริมาณ FBP1 mRNA คณะผู้วิจัยจึงได้ทำการทดสอบกลไกการออกฤทธิ์ของ WY-14643 ว่าผ่าน PPRE หรือไม่ โดยการสร้าง reporter plasmid ที่ประกอบด้วยจีน luciferase ภายใต้การควบคุมของ FBP1 promoter หรือมิวแตนท์ที่สูญเสีย PPRE เข้าสู่เซลล์ HepG2 ที่ได้รับและไม่ได้รับสาร WY-14643 และวัดระดับเอนไซม์ luciferase พบว่าเซลล์ที่ได้รับพลาสมิดที่มี wild type FBP1 promoter ถูกเหนี่ยวนำให้ระดับเอนไซม์ luciferase เพิ่มสูงขึ้นหลังการกระตุ้นด้วย WY-16463 ในขณะที่เซลล์ที่ได้รับพลาสมิดที่สูญเสีย PPRE ไม่มีการเพิ่มระดับเอนไซม์ luciferase หลังการกระตุ้นด้วย WY-16463 บ่งชี้ว่า WY-16463 ออกฤทธิ์กระตุ้นการแสดงออกของเอนไซม์ FBP1 ผ่าน PPRE บนโปรโมเตอร์จีน FBP1

คำสำคัญ: ฟรุคโตสบิสฟอสฟาเตส การสร้างน้ำตาลในตับ การควบคุมด้วยฮอร์โมน ตัวรับสัญญาณเปอร์ออกซิโซม การลอกรหัสจีน

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Regulation of human fructose-1,6-bisphosphtase gene expression by cAMP, dexamethasone and PPAR-α in HepG2 Cells

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Abstract

Fructose-1,6-bisphosphatase-1 (FBP1) catalyzes the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate which is a crucial intermediate in gluconeogenesis in liver. Aberrant expression of FBP1 can result in diabetes in animal models and human. Here we investigated the stimulatory effect of various ligands i.e., forskolin, dexamethasone, all-trans retinoic acid and peroxisome proliferator activated receptor-α agonist, WY-14643, on the abundance of human FBP1 mRNA in HepG2 cells. Exposure of HepG2 cells with forskolin, dexamethasone or all-trans retinoic acid did not affect expression of human FBP1 mRNA expression while treatment of HepG2 cells with 0.2 mM WY-14643 for 48 h and 72 h resulted in 2-fold increase of FBP1 mRNA concomitant with increased expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Furthermore, combined treatment of WY-14643 with dexamethasone further increased the expression of FBP1 mRNA. Nucleotide sequence analysis of human FBP1 gene revealed the presence of binding site for PPAR-α (PPRE) which mediates WY-14643 response, located at nucleotides -358/-346 and -212/-198. We next investigated the mechanism by which WY-14643 activates FBP1 expression by constructing the reporter plasmid consisting of luciferase gene driven by FBP1 promoter or mutant lacking PPRE. These constructs were then transfected into HepG2 cells following treatment with or without WY-14643, and the luciferase activity was measured. The results showed that WY-14643 increased luciferase activity in cells transfected with wild type FBP1 promoter while it did not affect luciferase activity in cells transfected with FBP1 promoter lacking PPRE. This result indicates WY-14643 exerts its stimulatory effect through PPRE in the promoter of FBP1 gene.

Keywords: fructose-1,6-bisphosphatase, gluconeogenesis, hormonal regulation, transcription, peroxisome proliferator-activated receptor-α (PPARα)

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Introduction

Hepatic glucose production is a pivotal biochemical process that supplies glucose to the blood circulation. Although both glycogenolysis and gluconeogenesis contribute to hepatic glucose production, the latter pathway accounts for up to 90% of total glucose release into the blood circulation[1]. Hepatic gluconeogenesis is enabled by the liver converting glycerol, lactate and alanine to glucose, thereby protecting the body from severe hypoglycemia during a prolonged starvation period. Hepatic gluconeogenesis is regulated by four rate-limiting step enzymes: pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBP) and glucose-6-phosphatase (G6Pase)[2]. Elevated plasma glucagon during fasting stimulates gluconeogenesis while elevated plasma insulin during feeding inhibits this pathway. The opposite action of these two hormones enables the body to maintain the plasma glucose concentration within a narrow range[3]. Deregulation of gluconeogenesis caused by impaired insulin secretion or hepatic insulin resistance results in overproduction of glucose from liver, leading to severe hyperglycemia [3].

FBP catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate[4]. There are two isozymes of FBP *viz*, liver FBP or FBP1 and muscle FBP or FBP2. FBP1 is exclusively expressed in liver and kidney where it supports gluconeogenesis. In contrast, FBP2 is highly expressed in skeletal muscle where it is thought to support glycogen synthesis. Accumulating evidence suggest that overexpression of FBP1 is associated with diabetes in several rodent models[5,6]. Because of this association, several FBP1 inhibitors have been developed in clinical trials as an anti-diabetic drug that can lower hepatic glucose production[7]. FBP1 activity has long been known to be regulated by fructose-2,6-bisphosphate (F2,6P), an allosteric inhibitor produced by the bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFK2/FBP2)[8,9]. Insulin stimulates 6PFK2 activity of 6PFK2/FBP2, resulting in an increased level of F2,6P, inhibiting FBP1 activity, while glucagon via cAMP, stimulates FBP2 activity, lowering the level of this allosteric inhibitor[10]. Although the positive effect of glucagon on FBP1 activity is apparent, it is not known whether glucagon or other hormones regulates FBP1 at the transcriptional level.

Here we investigated the effect of forskolin, dexamethasone, all-trans retinoic acids and peroxisome proliferator activated receptor-α (PPAR-α) agonist on the expression of mRNAs for FBP1 and other gluconeogenic enzymes. Among these ligands, we found that PPAR-α agonist WY-14643 was the only ligand that can induce expression of mRNAs for FBP1, PEPCK-C and G6Pase but not PC. The positive effect of PPARα agonist is mediated through the two peroxisome proliferator-responsive elements (PPRE), located in the promoter region of the human FBP1 gene.

Materials and Methods

Cell culture and ligand treatments

The human hepatocellular carcinoma cells, HepG2 (HB-8065) were routinely maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco), 28 mmol/l $NaHCO_3$ (Sigma-Aldrich), and 100 units/ml penicillin-streptomycin (Gibco) at 37°C in 5% CO_2 atmosphere. One day before ligand treatment, 5 x 10^4 cells were plated in 35 mm^2 dish containing the same medium except the concentration of glucose was decreased to 5 mM to mimic fasting glucose concentration. After 24 h, cells were treated with various ligands at different concentrations, i.e. 10 µM and 50 µM of forskolin (Sigma-Aldrich), 0.1 µM, 0.5 µM and 1 µM of dexamethasone (Sigma-Aldrich), 2 µM, 20 µM and 200 µM of 4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14643) (Sigma-Aldrich), 0.1 µM, 1 µM and 10 µM of all trans-retinoic acid (Sigma-Aldrich) or 0.1% (v/v) DMSO (vehicle control). Cells were then incubated at 37°C in 5% CO_2 for 6 h, 12 h, 24 h, 48 h and 72 h. All chemical compounds were dissolved in 0.1% DMSO. After treatment, cells were harvested for measuring gene expression.

Quantitative real time PCR

Total RNA was isolated from HepG2 cells using TRIzol®Reagent (Gibco), and the concentration of RNA was determined using NanoDrop (Thermoscientific). Reverse transcription was performed in a 20 µl-reaction mixture containing 2 µg of total RNA, 0.2 µg random hexamers, 1x ImProm-II™ reaction buffer, 3 mM $MgCl_2$, 0.5 mM dNTP mix and 1 µl of ImProm-II™ reverse transcriptase. The reaction was incubated at 25°C for 5 min before shifting to 42°C for 60 min and terminated at 70°C for 15 min. qPCR analysis for the expression of FBP1 and G6Pase mRNAs was performed in a 12 µl -reaction mixture containing 1x SYBR green Master mix (KAPPA Biosystems), 0.2 µM each of primer and 2 µl of cDNA. The thermal profiles consisted of an initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec and dissociation at 95°C for 1 min, 55°C for 30 sec and 95°C for 30 sec. qPCR analysis for the expression of PC and PEPCK-C mRNAs was performed using Taqman probes in which the 12-µl reaction contained 1x Probe Master mix (KAPA Biosystems), 1 µM each of primer, 0.5 µM of fluorogenic probe and 2 µl of cDNA. The thermal profiles consisted of an initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 1 min. The sequence of primers and fluorogenic probes for detecting FBP1, G6Pase, PC and PEPCK-C are shown in Table 1. Expression data were calculated from the cycle threshold (Ct) value using the ΔCt method of quantification. FBP1, G6Pase, PC or PEPCK-C expression was normalized with the expression of 18s ribosomal RNA and shown as relative gene expression.

Table 1 The sequence of primers and fluorogenic probes used for the quantitative real-time RT-PCR

Gene name	Forward primer	Reverse primer	Fluorogenic probe
hFBP1	5'-AGCCTTCTGAGA AGGATGCTC-3'	5'-GTCCAGCATGAAGCA GTTGAC-3'	-
hG6Pase	5'-GGGAAAGATAAA GCCGACCTAC-3'	5'-CAGCAAGGTAGATTC GTGACAG-3'	-
hPEPCK	5'-CCACAGCGGCTG CAGAACAT-3'	5'-GAAGGGCCGCATGG CAAA-3'	5'-AAGGCAAAATCATCA TGCATGACC-3'
hPC	5-GATGACTTCACAG CCCAG-3'	5'-GGGCACCTCTGTGT CCAG-3'	5'-CCCTGGTGGCCTGTA CCAAAGGG-3'
18s rRNA	5'-CGGCTACCACATC CAAGGAA-3'	5'-GCTGGAATTACCGC GGCT-3'	5'-TGCTGGCACCAGACTT GCCCTC-3'

Mutagenesis

The chimeric construct containing 400 nucleotides of human FBP1 promoter-luciferase reporter gene has previously been generated[11]. Mutations of two PPREs were generated using this plasmid as a template. Mutagenesis was carried out by PCR in a 50 µl-reaction mixture containing 1x cloned Pfu polymerase buffer [100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris HCl pH 8.8, 20 mM $MgSO_4$, 1% Triton ® X-100 and 1 mg/ml BSA], 0.2 mM dNTP, 125 ng of each mutagenic primer (Table 1), 100 ng of template, and 2.5 units of *Pfu* Turbo polymerase (Agilent Technologies). The PCR profile consisted of an initial denaturation at 95°C for 30s, followed by 20 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min and extension at 68°C for 8 min, and a final extension at 68°C for 8 min. The parental DNA template was digested by incubation of PCR mixture with 10 units of *DpnI* at 37°C overnight before 5 µl of the *DpnI* digested-PCR mixture were transformed into *Escherichia coli* DH5α. The clones containing corrected mutations were verified by nucleotide sequencing (Macrogen, South Korea).

Transient transfections and reporter gene assays

On the day of experiment, HepG2 cells were plated at a density of $1x10^5$ cells/cm^2 in into a 24-well culture plate containing 0.5 ml of antibiotic-free complete DMEM. After 24 h, cells were transfected with 0.2 pmol each of the hFBP1 promoter-luciferase reporter constructs and pRSV-β-gal vector expressing β-galactosidase using Lipofectamine™ 3000 reagent (Thermoscientific) according to the manufacturer's instructions. The transfected cells were incubated at 37°C with 5% CO_2 for 8 h as vehicle control. At 48 h after treatment, the transfected cells were harvested for measuring the luciferase activity using luciferase assay reagent (Promega) and the β-galactosidase activity using ONPG as substrate. The luciferase activity was normalized with the

β-galactosidase activity and presented as the relative luciferase activity.

Statistical analysis

All data are presented as the means ± standard deviations from three independent experiments. Statistical significance between samples was determined by using one way ANOVA analysis of variance, Sigma Stat 3.5.

Results

We first examined the effect of elevated cAMP, a second messenger for glucagon signaling using forskolin, an agent that activates adenyl cyclase[12] on the expression of mRNAs for FBP1 and cytosolic PEPCK (PEPCK-C). Exposure of HepG2 cells with 10 μM of forskolin did not affect expression of PEPCK-C, while exposure with 50 μM of forskolin resulted in 6-fold and 10-fold induction of PEPCK-C mRNA at 48 h and 72 h, respectively (Fig. 1A). Combined treatment with 50 μM of forskolin and 1 μM dexamethasone rapidly induced expression of PEPCK-C mRNA by 7-fold within 6 h. Chronic treatment of HepG2 cells with these two agents caused a decrease of PEPCK-C expression to 3-fold at 24 h before it sharply increased again at 48 h and 72 h, respectively (Fig. 1A). The positive effect of forkskolin on PEPCK-C mRNA expression is consistent with a previous study demonstrating that PEPCK-C gene is a cAMP responsive gluconeogenic enzyme[13]. Unlike PEPCK-C, FBP1 mRNA expression was not affected by treatment with forskolin at both concentrations or in combination with dexamethasone (Fig. 1B). These data indicate that FBP1 mRNA expression was not regulated by cAMP and dexamethasone.

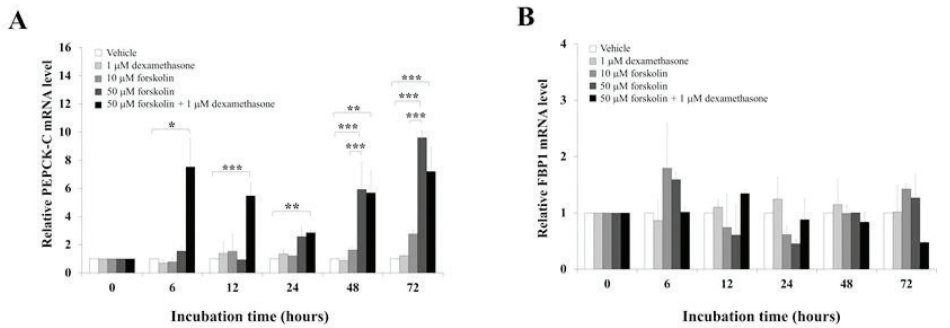


Figure 1 Effect of dexamethasone, forskolin and their combination on the expression of FBP1 and PEPCK-C mRNAs in HepG2 cells. HepG2 cells were cultured in complete medium containing 0, 10, 50 μM forskolin or 1μM dexamethasone or the combination of both ligands for 6, 12, 24, 48 and 72 h, respectively. Quantitative real time RT-PCR was performed to measure the abundance of PEPCK-C (A) or FBP1 (B) mRNAs. The values shown are means ± standard deviations of three independent experiments (n = 3). The statistical analysis was conducted by ANOVA test where *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

All trans-retinoic acid is another ligand which is known to stimulate gluconeogenesis, specifically through PEPCK-C14. To examine whether this ligand can also induce expression of mRNAs of FBP1 and other gluconeogenic enzymes, HepG2 cells were treated with 0.1, 1 and 10 μM of all-trans retinoic acid at various time points. As shown in Fig. 2A-C, short-term or long-term treatment of cells with all concentrations of all-trans retinoic acid did not affect expression of mRNAs for FBP1, PC or G6Pase. In contrast, treatment of cells with every concentration of all-trans retinoic acid resulted in a 2-fold increase of PEPCK-C expression. However, this induction was returned to the basal level after 12 h (Fig. 2D).

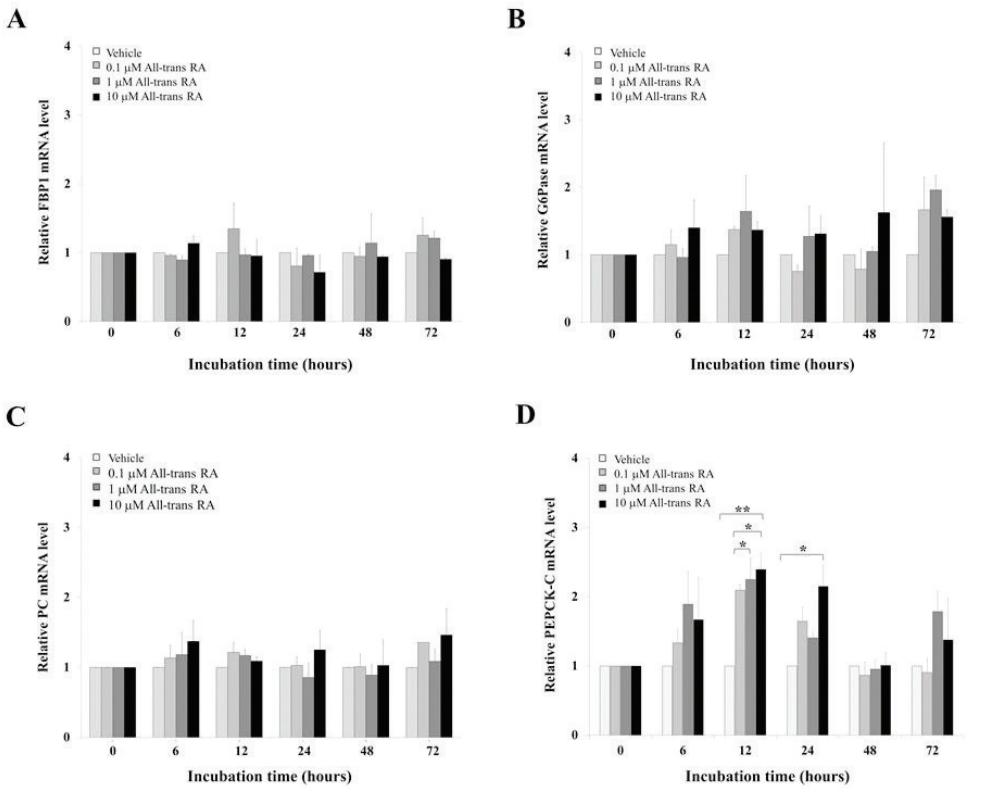
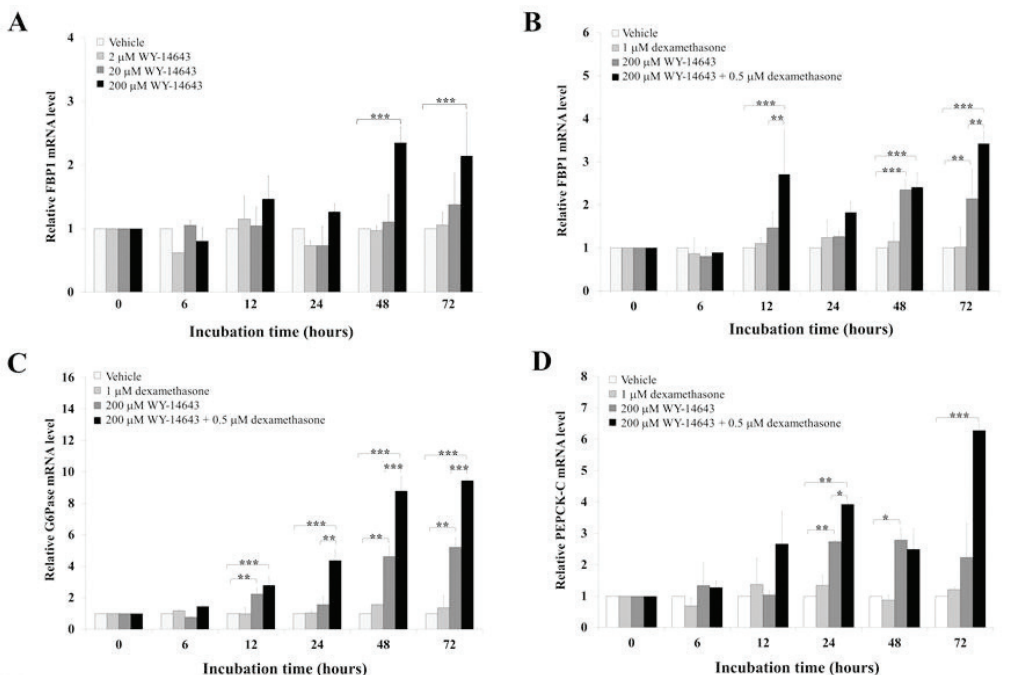


Figure 2 Effect of all-trans retinoic acid on the expression of gluconeogenic enzyme mRNAs in HepG2 cells. HepG2 cells were cultured in complete medium containing 0, 0.1, 1.0 and 10.0 μM all-trans retinoic acids for and 6, 12, 24, 48 and 72 h, respectively. Quantitative real time RT-PCR was performed to measure the abundance of FBP1 (A), G6Pase (B), PC (C) and PEPCK-C (D) mRNAs. The values shown are means ± standard deviation of three independent experiments (n = 3). The statistical analysis was conducted by ANOVA test where *$p < 0.05$, **$p < 0.01$.

PPARα, a nuclear receptor that has recently been shown to stimulate fatty acid oxidation during prolonged fasting when its expression is highly induced[15]. To examine whether this nuclear receptor coordinates gluconeogenesis by modulating FBP1 expression and other gluconeogenic enzymes, HepG2 cells were treated with various concentrations of PPARα agonist, WY-14643. As shown in Fig. 3A, low concentrations of WY-14643 did not affect expression of FBP1 mRNA while treatment with 0.2 mM WY-14643 resulted in 2.5-fold induction of FBP1 mRNA at both 48 and 72 h. Combined treatment of WY-14643 with 0.5 μM dexamethasone caused significant increase of FBP1 mRNA expression especially at 12 h, 48 h and 72 h (Fig. 3B). WY-14643 can also induce expression of G6Pase as early as 12 h and continued to increase at 48 h and 72 h, respectively, and dexamethasone produced an additive effect on WY-14643-induced expression of G6Pase at 24, 48 and 72 h, respectively (Fig. 3C). Similar to FBP1, short term treatment of HepG2 cells with WY-14643 alone at 6 h and 12 h did not affect the expression of PEPCK-C mRNA while exposure of this ligand for 24 h, 48 h and 72 h resulted in 3-fold induction of PEPCK-C mRNA. Dexamethasone also produced an additive effect on WY-14643-induced expression of PEPCK-C mRNA (Fig. 3D). In contrast, neither treatment of W-14643 alone nor in combination with dexamethasone increased expression of PC mRNA (Fig. 3E).



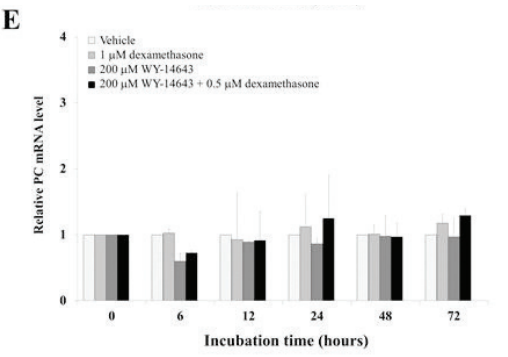


Figure 3 Effect of WY-14643 on the expression of gluconeogenic mRNAs in HepG2 cells. (A) Expression of FBP1 mRNA in HepG2 cells was treated with 0, 2, 20 and 200 μM WY-14643 for 6 h, 12 h, 24 h, 48 h and 72 h, respectively. Expression of FBP1 (B), PEPCK-C (C), G6Pase (D) and PC (E) in HepG2 cells treated with 0, 1 μM dexamethasone, 200 μM WY-14643 or 200 μM WY-14643 and 0.5 μM dexamethasone for 6 h, 12 h, 24 h, 48 h and 72 h, respectively. The statistical analysis was conducted by ANOVA test where $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

The positive effect of PPARα agonist on FBP1 expression prompts us to further examine the regulatory mechanism how this ligand can stimulate expression of human FBP1 gene. Typically, PPARα exerts its transcriptional activation upon binding to the peroxisome-proliferator activated receptor responsive element (PPRE) of its target promoters. Using JASPAR16 and Dragon PPRE spotter v. 2.0 (http://www.cbrc.kaust.edu.sa/ppre) tools for predicting cis-acting elements of a eukaryotic promoter, two PPREs were identified between nucleotides -358/-346 (PPRE1: 5'-AGGTGACAGGCCA-3': sense strand) and -212/-198 (PPRE2: 5'- AGTTCAGAA AGGTTA-3': antisense strand) of human FBP1 promoter (Fig. 4A). The underlines indicate two tandem consensus hexanucleotides, AGGTCA separated by one nucleotide (known as DR1) or three nucleotides (DR3), typical of nuclear receptor binding sites[17]. To examine whether WY14643-induced FBP1 mRNA expression is mediated through these two PPREs, we transiently transfected the human FBP1 promoter-luciferase construct (Fig 4B) into HepG2 cells followed by treating the transfected cells with or without WY-14643 and dexamethasone for 48 h. As shown in Fig. 4C, HepG2 cells transfected with the human FBP1 promoter-luciferase construct showed a 2-fold increase in the luciferase activity following treatment with the ligands. Substitution of PPRE1 or PPRE2 with a non-related sequence in human FBP1 promoter abrogated ligand activation, suggesting that WY14643-induced FBP1 mRNA expression observed in Fig. 3 is likely mediated through these two PPREs.

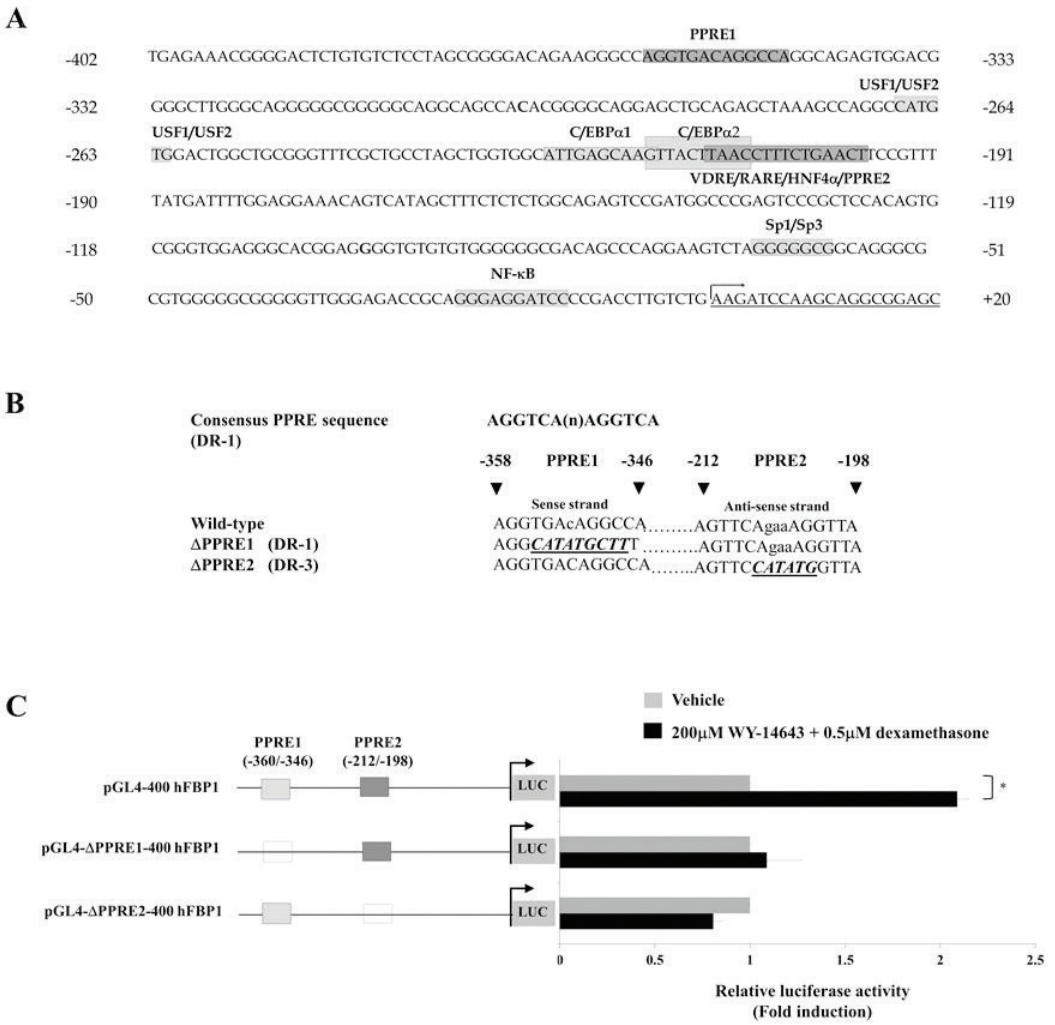


Figure 4 Identification of WY-14643 responsive element in the promoter of hFBP1 gene. (A) Nucleotide sequence of human FBP1 promoter and the transcription factor binding sites. (B) Comparison of consensus PPRE (DR1) and PPREs located at nucleotides -358/-346 (PPRE1) and -212/-198 (PPRE2) in hFBP1 promoter of hFBP1 gene. Also shown are the nucleotide substitutions introduced in the PPRE mutants (ΔPPRE1 and ΔPPRE2). (C) Expression of luciferase in HepG2 cells transfected with luciferase reporter plasmid driven by wild type hFBP1 promoter or mutants harboring PPRE1 or PPRE2 substitution under basal (vehicle) or in the presence of 200 μM WY-14643 and 0.5 μM dexamethasone for 48 h. Relative luciferase activity obtained from cells transfected with mutated hFBP1 promoter- luciferase and maintained in culture medium with or without WY-14643 was relative to those transfected with WT FBP1 promoter maintained in culture medium without WY-14643, which as arbitrarily set as 1. The values shown are means ± standard deviation of three independent experiments (n = 3). The statistical analysis was conducted by ANOVA test where $*p < 0.05$.

Discussion

Hepatic gluconeogenesis is a crucial *de novo* glucose synthesis pathway that enables liver to convert non-carbohydrate precursors to glucose5. Tight regulation of this pathway is pivotal to maintain systemic glucose homeostasis. Alterations of various hormones in plasma during starvation and feeding cycles dramatically influence this pathway either by activation or repression[1]. In this study, we investigated whether or not various ligands including forskolin, dexamethasone, all-trans retinoic acid and PPARα agonist, WY-14643 can activate expression of mRNAs encoding FBP1 and other gluconeogenic enzymes in human hepatocytes.

Although cAMP is well known to increase FBP1 activity by lowering level of its allosteric inhibitor, F2,6P[8,9], increasing intracellular cAMP by forskolin treatment did not affect expression of human FBP1 mRNA at both short and long terms. The lack of cAMP response on the human FBP1 gene differs from the rat FBP1 gene in which treatment of rat hepatocytes with the same agent results in a sharp increase of FBP1 mRNA levels within 12 h to 24 h. The positive effect of cAMP on rat FBP1 expression is attributed to the presence of CRE in the promoter of rat FBP1 gene[18] which is not the case for human FBP1 promoter[11]. Although dexamethasone, a synthetic glucocorticoid has been shown to increase hepatic gluconeogenesis[1,5], this hormone did not regulate expression of human FBP1 mRNA. The lack of response of human FBP1 gene to this hormone is similar to the study performed in mouse in which treatment of hepatocytes with this hormone resulted in only a slight change of mouse FBP1 mRNA[19].

The peroxisome proliferator activated receptor-α (PPAR-α) is highly induced during prolonged fasting[20]. PPAR-α enhances gene transcription by forming a heterodimer with retinoid-X-receptor (RXR), prior to binding to the responsive element of the target genes. In the presence of ligand or agonist, PPARα-RXR complex recruits co-activator to the target promoters and activates transcription[21]. The well-known metabolic targets of PPARα are the enzymes that regulate fatty acid oxidation. This mechanism enables fat oxidative tissues to produce ATP from lipids during carbohydrate deprivation conditions [20]. The association between PPARα and glucose homeostasis was first reported in PPARα (-/-) null mice which display a symptom of fasting hypoglycemia, partly attributed to the depletion of liver glycogen and impaired gluconeogenesis[22]. In our study, we found that PPAR-α agonist, WY-14643 induced expression of mRNA for human FBP1 at 48 h and 72 h while co-treatment of this ligand with dexamethasone enhanced expression of FBP1 mRNA. WY-14643 also induced expression of mRNAs for PEPCK-C and G6Pase, however the degree of induction was varied, with G6Pase being the most inducible followed by PEPCK-C and FBP1, respectively. Our results provide an

explanation for the observation of lower expression of FBP1, G6Pase and PEPCK-C in PPARα (-/-) null mice[23-25]. We were able to identify that the mechanism of WY-14643-mediated activation of FBP1 expression is mediated through the two PPREs in the promoter region of human FBP1 gene. It is noted that PPARα ligand-induced expression of PPARα target genes is typically mediated through the two direct repeat sequences, AGGTCA separated by one nucleotide known as DR126. In the case of the human FBP1 gene only PPRE1 resembles DR1 while PPRE2 resembles DR3 (two direct repeats separated by three nucleotides). Interestingly, this PPRE2 in human FBP1 gene appears to overlap with the vitamin D responsive element (VDRE) that directs vitamin D response in leukocytes[27]. Dexamethasone alone cannot induce expression of FBP1 mRNA while it can amplify WY-14643-induced expression of FBP1 mRNA. This additive effect of dexamethasone on WY-14643-induced expression was also observed with PC, PEPCK and G6Pase (Fig. 3). This is not entirely surprising because glucocorticoid receptors can interact with some members of the nuclear receptor family[28]. Specifically, Bougarne *et al.*[29] have shown that the glucocorticoid receptors can interact with PPARα in a synergistic manner and regulate expression of NF-κb expression. It is possible that a higher expression level of PPARα and its ligands combined with increased level of glucocorticoids during fasting ensures maximal activation of FBP1 expression. Although all-trans retinoic acid has been reported to regulate expression of PEPCK-C [14], this ligand did not appear to regulate expression of FBP1, PC and G6Pase mRNAs. Our result was consistent with the previous study demonstrating that the vitamin-A deficient mice displayed a marked reduction of mRNAs for hepatic PEPCK-C but not for hepatic FBP1 during fasting period[30].

Pirinixic acid or PPARα agonist, WY-14643 is currently under investigation for the prevention or treatment of cardiovascular disease[31]. This drug acts by increasing fatty acid oxidation thus lowering triglycerides in circulation which reduces LDL-induced atherosclerosis[31]. However, the caution should be taken because this drug can induce expression of FBP1, PEPCK-C and G6Pase which may induce excessive hepatic gluconeogenesis, contributing to hyperglycemia.

Conclusions

We show that not all mRNAs for gluconeogenic enzymes respond to forskolin, dexamethasone, all-trans retinoic acid and PPARα ligand in the same way. Forskolin strongly induced expression of PEPCK-C mRNA but not FBP1 mRNA while dexamethasone increased expression of PEPCK-C and G6Pase but not PC and FBP1. All-trans retinoic acid only induced expression of PEPCK-C while PPARα ligand increased expression of FBP1, PEPCK-C and G6Pase but not PC. It is also noted that some ligands can induce expression of the mRNAs for certain gluconeogenic enzymes more robustly than others.

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