World Journal of Gastroenterology

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DOI: 10.3748/wjg.v20.i42.15727

World J Gastroenterol 2014 November 14; 20(42): 15727-15735
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

RESEARCH REPORT

GW4064, a farnesoid X receptor agonist, upregulates adipokine expression in preadipocytes and HepG2 cells

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Supported by Guangdong Provincial Science and Technology Projects, No. 2011B050400009; and Scientific Research Projects of Hubei Province Education Department, No. B2014055
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Received: March 14, 2014 Revised: June 13, 2014
Accepted: July 11, 2014
Published online: November 14, 2014

Abstract

AIM: To investigate the effect of GW4064 on the expression of adipokines and their receptors during differentiation of 3T3-L1 preadipocytes and in HepG2 cells.

METHODS: The mRNA expression of farnesoid X receptor (FXR), peroxisome proliferator-activated receptor-gamma 2 (PPAR-γ2), adiponectin, leptin, resistin, adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2), and the long isoform of leptin receptor (OB-Rb) and protein levels of adiponectin, leptin, and resistin were determined using fluorescent real-time PCR and enzyme linked immunosorbent assay, respectively, on days 0, 2, 4, 6, and 8 during the differentiation of 3T3-L1 preadipocytes exposed to GW4064. Moreover, mRNA expression of AdipoR2 and OB-Rb was also examined using fluorescent real-time PCR at 0, 12, 24, and 48 h in HepG2 cells treated with GW4064.

RESULTS: The mRNA expression of FXR, PPAR-γ2, adiponectin, leptin, resistin, AdipoR1, AdipoR2, and OB-Rb and protein levels of adiponectin, leptin, and resistin increased along with differentiation of 3T3-L1 preadipocytes ($P < 0.05$ for all). The mRNA expression of FXR, PPAR-γ2, adiponectin, leptin, and AdipoR2 in 3T3-L1 preadipocytes, and AdipoR2 and OB-Rb in HepG2 cells was significantly increased after treatment with GW4064, when compared with the control group ($P < 0.05$ for all). A similar trend was observed for protein levels of adipokines (including adiponectin, leptin and resistin). However, the expression of resistin, AdipoR1, and OB-Rb in 3T3-L1 cells did not change after treatment with GW4064.

CONCLUSION: The FXR agonist through regulating, at least partially, the expression of adipokines and their receptors could offer an innovative way for counteracting the progress of metabolic diseases such as nonalcoholic fatty liver disease.

Key words: Farnesoid X receptor; Adipokines; Adipokine receptors; 3T3-L1 cells; HepG2 cells; Nonalcoholic fatty liver disease

Core tip: Our study emphasizes for the first time the effect of GW4064, a synthetic farnesoid X receptor (FXR) agonist, on the expression of adipokines and their receptors, and indicates that the way FXR agonist may act in the progress of nonalcoholic fatty liver disease (NAFLD) is at least partially through regulat-

ing the expression of adipokines and their receptors. Therefore, our data provide a further theoretical basis for using an FXR agonist in the therapeutic approach of NAFLD.

Xin XM, Zhong MX, Yang GL, Peng Y, Zhang YL, Zhu W. GW4064, a farnesoid X receptor agonist, upregulates adipokine expression in preadipocytes and HepG2 cells. *World J Gastroenterol* 2014; 20(42): 15727-15735 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i42/15727.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i42.15727

INTRODUCTION

In light of the rapidly increasing incidence of obesity, nonalcoholic fatty liver disease (NAFLD) is becoming one of the most common chronic liver diseases worldwide[1], affecting 20%-40% of the general population in industrialized countries[2]. NAFLD encompasses the entire spectrum of fatty liver disease in individuals without significant alcohol consumption, ranging from hepatic steatosis to more severe situations like nonalcoholic steatohepatitis (NASH) and cirrhosis. NAFLD begins with hepatic steatosis, and in 12%-40% can progress to NASH[3]. NASH is characterized by the presence of hepatocyte ballooning and apoptosis, inflammation and pericellular fibrosis. NASH may further progress to liver cirrhosis, eventually leading to hepatocellular carcinoma and liver failure. In late stages of NASH liver transplantation may be needed[4,5]. Pathogenesis of NAFLD remains poorly understood[6]. Presently, the predominant treatment for NAFLD consists of weight loss with lifestyle modifications[7] because no drugs have yet been approved by international agencies. There is an urgent need to develop novel therapeutics for treatment of this common and potentially severe disease.

Farnesoid X receptor (FXR), a member of the nuclear receptor superfamily of ligand activated receptors, is mainly expressed in the liver, intestine, kidneys and adrenal glands, with less expression in the adipose tissue and heart[8-10]. Initially, FXR was thought to have an important role in regulating bile acid metabolism[11]. A number of studies have since proved that FXR has many other functions, especially for regulating metabolic homeostasis. Numerous studies have demonstrated that activation of FXR by its selective agonist can reverse biochemical and hormonal dysfunction of NAFLD. Firstly, FXR is able to improve peripheral insulin sensitivity in striated muscle and adipose tissue, and control glucose homeostasis through regulation of gluconeogenesis and glycogenolysis in the liver[12-15]. Secondly, FXR is a key regulator of lipid metabolism. Several studies agree that FXR activation can alleviate accumulation of triglycerides, oxidative stress and lipid peroxidation in the liver by reducing serum lipid levels[16-19]. Lastly, inflammation and fibrosis in the liver are the main histopathological features of NASH, and several studies have demonstrated that FXR agonists can antagonize hepatic inflammatory processes and exert protective effects against liver fibrosis[20-22]. Li *et al*[23] also demonstrated that GW4064 could inhibit ET-1-mediated activation of the Rho/Rho-associated kinase (ROCK) pathway in activated hepatic stellate cells (HSCs), which could represent a new mechanism contributing to the anti-cirrhotic effects of FXR ligands. It is noteworthy that hepatic FXR expression declines in patients with NAFLD[24]. All these data suggest that FXR could represent an interesting therapeutic approach for NAFLD.

Adipokines, secreted by the white adipose tissue, which is an important endocrine organ, take part in the regulation of insulin resistance, energy metabolism and metabolic syndromes. They are also thought to play an important role in the pathogenesis of NAFLD[25,26]. Rizzo *et al*[15] demonstrated that FXR was expressed in the adipose tissue, where its activation promoted the process of adipocyte differentiation. However, the effects of FXR activation on the adipokines and their receptors are not yet fully understood. In the present study, we investigated whether FXR controls the expression of adipokines and their receptors directly or indirectly. We studied the effects of GW4064, an FXR selective agonist, on adipokines (including adiponectin, leptin and resistin) and their receptors [including adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2) and the long isoform of leptin receptor OB-Rb] during the differentiation of 3T3-L1 preadipocytes, and on AdipoR2 and OB-Rb in HepG2 cells.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), trypsin and fetal bovine serum (FBS) were acquired from Hyclone (Waltham, MA, United States). GW4064 was obtained from Tocris Bioscience (Bristol, United Kingdom). Dimethylsulfoxide (DMSO), oil red O staining, insulin, dexamethasone, and 1-methyl-3-isobutyl-xanthin (IBMX) were purchased from Sigma (Saint Louis, MO, United States). RNAiso Reagent, PrimeScript RT reagent kit with gDNA eraser and SYBRPremix Ex Taq™ Kit were acquired from TAKARA (Dalian, China). Florescent real-time quantitative RT-PCR was performed using a Roche LightCycler480 (Roche Diagnostics Ltd, Lewes, United Kingdom), and PCR primers were designed by Invitrogen (Shanghai, China). Mouse resistin, adiponectin and leptin enzyme linked immunosorbent assay (ELISA) kits were purchased from Yajikit (Shanghai, China) and the microplate reader was a TECAN F50 (Männedorf, Switzerland).

Cell culture and differentiation

3T3-L1 preadipocytes and HepG2 cells (The Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM supplemented with

Table 1 Sequences of primers for reverse transcription and real-time polymerase chain reaction amplification

Species	Gene	Forward primer	Reverse primer	Amplification (bp)
Rat	*FXR*	GCTGTGTGTTGTCTGTGGAGA	GGCGTTCTTGGTAATGCTTC	103
	PPARγ2	AGCTCTACAACAGGCCTCAT	TTGTGGATCCGGCAGTTAAG	190
	Adiponectin	CCTGGCCACTTTCTCCTCAT	ACGTCATCTTCGGCATGACT	132
	leptin	CCTGTGGCTTTGGTCCTATC	ATACCGACTGCGTGTGTGAA	128
	resistin	AGCTGTGGGACAGGAGCTAA	GGAGGGGAAATGAAAGGTTC	96
	AdipoR1	TCCACACAGAGACTGGCAAC	TTGGTCTCAGCATCGTCAAG	87
	AdipoR2	TGTTTGCCACCCCTCAGTAT	AGCCAGCCTATCTGCCCTAT	139
	OB-Rb	AGCTAGGTGTAAACTGGGACA	GCAGAGGCGAATCATCTATGAC	159
	GAPDH	ACCCCCAATGTGTCCGTCGT	AGCCCAAGATGCCCTTCAGTGG	118
Human	*AdipoR2*	TGTTTGCCACCCCTCAGTAT	CAGCCTATCTGCCCTATGGT	136
	OB-Rb	TACTTTGGAAGCCCCTGATG	AAGCACTGAGTGACTGCACG	246
	GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA	145

FXR: Farnesoid X receptor; PPAR-γ2: Peroxisome proliferator-activated receptor-gamma 2; AdipoR1: Adiponectin receptor 1; AdipoR2: Adiponectin receptor 2; OB-Rb: Long form of the leptin receptor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 ℃ in a 5% CO_2 humidified atmosphere.

3T3-L1 preadipocytes were differentiated two days after inducing confluence by exposing them to a differentiation mixture (DIM) containing 5 μg/mL insulin, 0.5 mmol/L IBMX, and 1 μmol/L dexamethazone in 10% FBS-supplemented DMEM for 48 h. On day 2, cells were cultured in DMEM containing 10% FBS and 5 μg/mL insulin for 48 h. On day 4 and thereafter, DMEM containing 10% FBS only was subsequently replaced every 48 h. In general, by day 8, 80%-90% of preadipocytes differentiated into adipocytes as determined by lipid accumulation visualized by Oil Red O staining.

In the 3T3-L1 adipocytes, the treatment group received 5 μmol/L of GW4064 on day 0 as the differentiation was beginning; the control group received an equal volume of DMSO at the same time. In the HepG2 cells, the treatment group received 5 μmol/L of GW4064 for 0, 12, 24, or 48 h after the cells had been serum-deprived for 24 h; the control group received an equal volume of DMSO at the same time.

RNA extraction and cDNA synthesis

Total RNA was isolated from 3T3-L1 cells in both the treatment and control groups using RNAiso Reagent on 0, 2, 4, 6, and 8 d after the start of the differentiation process; total RNA was isolated from HepG2 cells at 0, 12, 24, 48 h after addition of GW4064. cDNA was synthesized from 1 μg of total RNA using PrimeScript RT reagent kit with gDNA eraser.

Real-time PCR

For real-time PCR, 100 ng of template was used in a 20 μL reaction system containing 0.2 μmol/L of each primer and 10 μL of 2 × SYBRPremix Ex Taq™. Samples were incubated in the LightCycler for an initial denaturation at 95 ℃ for 30 s, followed by 40 PCR cycles, each consisting of 95 ℃ for 5 s and 50 ℃ for 20 s. The oligonucleotide primers for the study are shown in Table 1. Melting curve profiles, which depict cooling of the sample to 65 ℃ for 15 s and heating slowly to 95 ℃ with continuous measurement of fluorescence, were produced at the end of each PCR to confirm amplification of specific transcripts. The mean value of the duplicates for each sample was calculated and expressed as the cycle threshold (CT). The amount of mRNA was normalized according to that of the endogenous control (GAPDH). The ΔCt values were calculated in every sample for the target gene as follows: $Ct_{\text{target gene}}$ - $Ct_{\text{reference gene}}$ with GAPDH as the reference gene. Relative expression (ΔΔCt) was calculated as ΔCt of the test group minus ΔCt of the control group and then presented as $2^{-\Delta\Delta Ct}$.

ELISA

To evaluate the protein levels of adipokines, we used ELISA kits to detect the supernatant collected during the differentiation of 3T3-L1 cells and measured the concentrations of adiponecin, leptin and resistin. ELISA was performed according to the manufacturer's instructions. Standard curves and sample concentrations were calculated based on absorbance (OD) values at 450 nm, as measured using a microplate reader.

Statistical analysis

All data are expressed as mean ± SD. Student's *t* test or one-way ANOVA was used to determine significant differences between groups. In all statistical comparisons, a *P* value less than 0.05 was considered statistically significant. All experiments were conducted at least three times. All analyses were performed using SPSS version 13.0.

RESULTS

Expression of FXR and PPARγ2 mRNAs during differentiation of 3T3-L1 adipocytes after GW4064 treatment

FXR mRNA expression increased 2.17-fold by the second day, reached the peak on the fourth day (2.66-fold), and was 2.31-fold greater than in preadipocytes on the eighth day ($F = 22.238$, $P < 0.001$) (Figure 1A). PPARγ2 mRNA level gradually increased along with maturation and was 2.96-fold greater than in preadipocytes on the

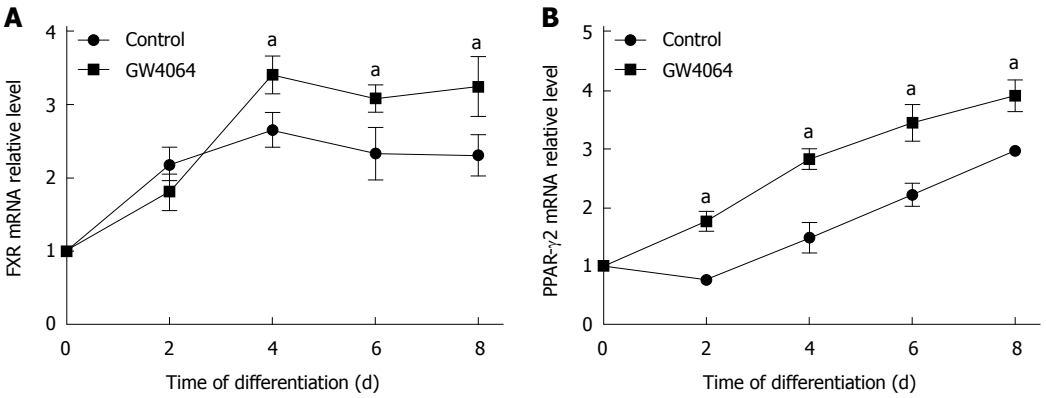

Figure 1 GW4064 (5 μmol/L) stimulates farnesoid X receptor (A) and peroxisome proliferator-activated receptor-gamma 2 (B) mRNA expression during the differentiation of 3T3-L1 preadipocytes. GW4064 was added on day 0. Total RNA was extracted and subjected to fluorescent real-time PCR as described in Section 2. FXR and PPAR-γ2 mRNA levels were determined according to normalized GAPDH expression and compared to untreated control cells (100%). Data represent mean ± SD of three independent experiments. $^{a}P < 0.05$ *vs* the control group. Error bars = mean ± SD. FXR: Farnesoid X receptor; PPAR-γ2: Peroxisome proliferator-activated receptor-gamma 2.

eighth day ($F = 128.900$, $P < 0.001$) (Figure 1B).

Subsequently, our experiment focused on the effect of FXR agonist on the process of adipocyte differentiation: the 3T3-L1 cells were treated with 5 μmol/L GW4064, since when they were preadipocytes. We found that FXR expression was maximal on the eighth day (1.40-fold greater than in the control group, $P < 0.05$) (Figure 1A). Finally, we observed that GW4064 increased PPARγ2 mRNA expression at the maturation of preadipocytes (1.32-fold greater than in the control group, $P < 0.01$) (Figure 1B).

Protein and mRNA levels of each adipokine and their corresponding receptor increase during the differentiation of 3T3-L1 adipocytes after GW4064 treatment

Gene expression of adipokines (including adiponectin, leptin and resistin) and their corresponding receptors (including AdipoR1, AdipoR2 and OB-Rb) increased along with the differentiation process ($P < 0.05$ for all), and all, except adiponectin, could be detected in the 3T3-L1 preadipocytes before the differentiation (Figure 2). Quantitative real-time PCR analysis revealed that GW4064 treatment prompted 1.27-, 1.19-, and 1.45-fold increases in mRNA expression of adiponectin, leptin and AdipoR2, respectively ($P < 0.01$) (Figure 2A, B, E). However, there were no changes in the expression of resistin, AdipoR1 or OB-Rb (Figure 2C, D, F). Furthermore, protein levels of adiponectin, leptin and resistin followed the same trend as their gene expression. We also observed that the levels of adiponectin and resistin secretion were greater than that of leptin (Table 2).

GW4064 raises AdipoR2 and OB-Rb gene expression in a time-dependent manner in HepG2 cells

We further tested whether GW4064 could affect the adipokine receptors in a targeted organ such as the liver. For this purpose, we detected mRNA expression of AdipoR2 and OB-Rb in the HepG2 cells treated with GW4064 (5 μmol/L) for 0, 12, 24, or 48 h. We considered only AdipoR2 in our study because it is mainly expressed in the liver, whereas AdipoR1 is mainly expressed in skeletal muscles. After 48 h of GW4064 treatment, AdipoR2 and OB-Rb mRNA expression increased 1.62- and 1.36-fold, respectively, and the increase was time-dependent ($P < 0.001$) (Figure 3A, B).

DISCUSSION

Adiponectin is an insulin-sensitizing hormone, which is almost exclusively secreted by the adipose tissue. Adiponectin is the only adipokine whose serum level is inversely correlated with body mass index (BMI) and adipose tissue volume. Adiponectin exhibits anti-atherogenic, anti-diabetic and anti-inflammatory properties, and plays a key role in slowing down the progression of NAFLD[27-29]. Adiponectin achieves these effects through two distinct receptors, AdipoR1 and AdipoR2, which play different biological roles[30,31]. AdipoR1, which is ubiquitously expressed and mostly abundant in the skeletal muscle, regulates *de novo* lipogenesis and fatty acid (FA) oxidation through the activation of AMP-activated protein kinase (AMPK). AdipoR2, which is mainly expressed in the liver, suppresses inflammation and oxidative stress through a peroxisome proliferator activated receptor[32,33]. It is noteworthy that decreasing levels of plasma adiponectin and hepatic AdipoR2, not AdipoR1, were found in patients with NAFLD or NASH[34,35]. Low adiponectin levels are correlated with insulin resistance and liver steatosis[36]. Moreover, impaired expression of adiponectin receptors may be associated with adiponectin resistance. Thus, we hypothesized that both plasma adiponectin levels and hepatic expression of adiponectin receptors might play roles in the development of NAFLD. In the present study, real-time PCR and ELISA analysis revealed that GW4064, a highly selective FXR agonist, increased gene expression and secretion of adiponectin in 3T3-L1 cells *in vitro*. The mRNA expres-

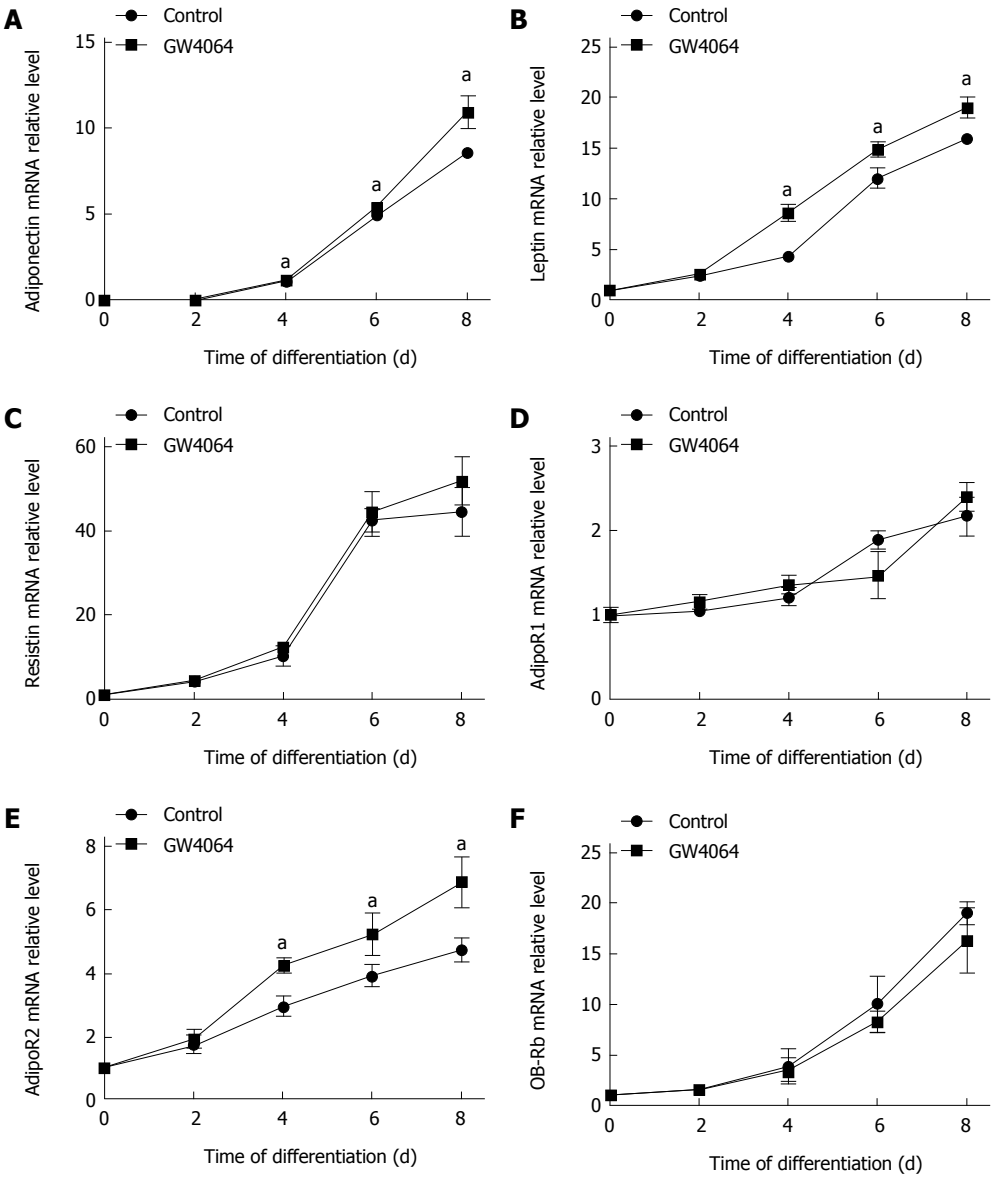

Figure 2 GW4064 (5 μmol/L) stimulates adiponectin (A), leptin (B), adiponectin receptor 2 (E) mRNA expression, but did not change expression of resistin (C), adiponectin receptor 1 (D), or long form of the leptin receptor (F) during the differentiation of 3T3-L1 preadipocytes. GW4064 was added on day 0. Total RNA was extracted and subjected to fluorescent real-time PCR as described in Section 2. The mRNA levels of target genes were normalized to GAPDH expression and compared with untreated control cells (100%). Data represent mean ± SD of three independent experiments. [a]$P < 0.05$ *vs* the control group. Error bars = mean ± SD. AdipoR1: Adiponectin receptor 1; AdipoR2: Adiponectin receptor 2; OB-Rb: Long form of the leptin receptor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Table 2 Protein levels of adiponectin, leptin and resistin as detected by enzyme linked immunosorbent assay (mean ± SD)

Adipokines	Group	Differentiation time (d)				
		0	2	4	6	8
Adiponectin (μg/L)	Control	0	0	73.16 ± 1.14	75.13 ± 0.79	80.12 ± 3.88
	GW4064	0	0	74.98 ± 1.14	94.64 ± 4.54[a]	96.30 ± 1.72[a]
Leptin (pg/mL)	Control	527.75 ± 7.30	579.22 ± 2.62	601.92 ± 11.65	647.34 ± 3.47	652.63 ± 4.73
	GW4064	527.75 ± 7.30	590.57 ± 11.65	629.93 ± 7.97[a]	686.69 ± 7.30[a]	741.19 ± 3.47[a]
Resistin (μg/L)	Control	58.97 ± 0.21	64.20 ± 0.25	72.10 ± 0.47	82.36 ± 0.83	94.31 ± 2.31
	GW4064	58.97 ± 0.21	64.06 ± 0.08	72.18 ± 0.31	84.26 ± 2.19	92.41 ± 0.13

[a]$P < 0.05$ *vs* untreated cells.

sion of hepatic AdipoR2 was also elevated in a time-dependent manner. Therefore, the activation of FXR could improve NAFLD by directly increasing serum adiponectin levels and enhancing the effects of adiponectin

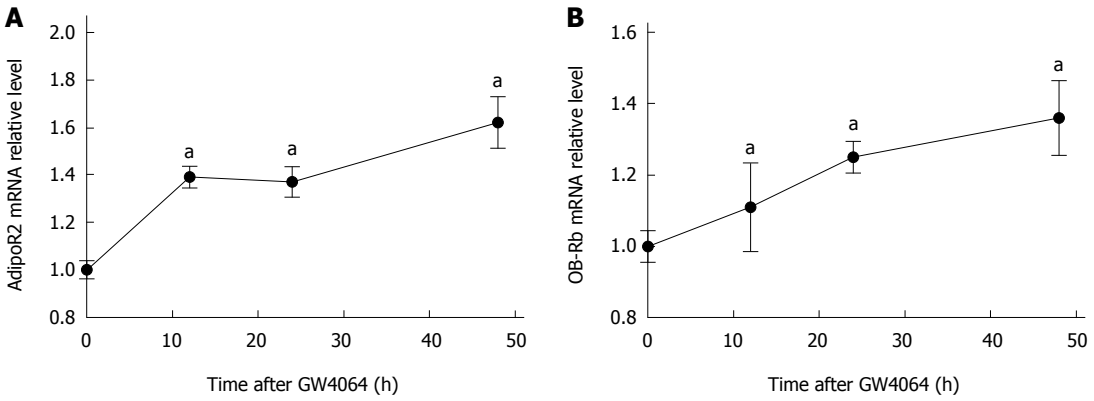

Figure 3 GW4064 (5 μmol/L) stimulates adiponectin receptor 2 (A) and long form of the leptin receptor (B) mRNA expression in HepG2 cells in a time-dependent manner. HepG2 cells were serum-deprived 24 h before GW4064 was added. Total RNA was extracted and subjected to fluorescent real-time PCR as described in Section 2. AdipoR2 and OB-Rb mRNA levels were normalized to GAPDH expression and compared to cells at 0 h (100%). Data represent mean ± SD of three independent experiments. $^{a}P < 0.05$ *vs* the cells at 0 h. Error bars = mean ± SD. AdipoR2: Adiponectin receptor 2; OB-Rb: Long form of the leptin receptor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

on the targeted organ by indirectly increasing hepatic AdipoR2 expression. Furthermore, our study showed that the mRNA expression of AdipoR2, but not AdipoR1, increased after GW4064 treatment in 3T3-L1 adipocytes. Previous reports have shown that adiponectin also exerts autocrine or paracrine effects on adipocytes themselves. Overexpression of adiponectin accelerates adipogenesis and generates more numerous and larger lipid droplets in differentiated adipocytes[37]. Therefore, GW4064 could also augment the autocrine and paracrine effects of adiponectin by elevating the expression of AdipoR2 in 3T3-L1 cells, leading to acceleration of 3T3-L1 fibroblast differentiation and increased adiponectin secretion.

Leptin is a product of the *OB* gene and is mainly released by the white adipose tissue in a mass-dependent manner. Leptin represses food intake, promoting energy expenditure by acting at the hypothalamus level, and stimulating the anorexigenic pathways[38,39]. Leptin activates its receptors at the cell membrane level in order to exert its properties. Leptin receptors (OB-R) are subdivided into long, short, and soluble isoforms, and can be found throughout the body, but are especially abundant in the brain and liver[40,41]. The short isoform (OB-Ra) has limited signaling activity, whereas the long isoform (OB-Rb) mediates almost all of the biological effects of leptin by activating the JAK2/STAT3 protein kinase signal transduction cascade[42]. The role of leptin in NAFLD patients is still debated because of conflicting results. Some reports indicate that leptin participates in the pathogenesis of NAFLD by amplifying inflammation and fibrogenesis through upregulation of pro-inflammatory and profibrogenic cytokines[43]. Leptin also inhibits insulin activities[44]. Serum leptin levels are also higher in patients with NAFLD than in healthy subjects[45,46] and are positively correlated with NAFLD severity. As a result, these patients develop leptin resistance. In contrary, some reports hold the opposite idea that leptin is a protective factor for NAFLD. Schwartz *et al*[47] demonstrated that leptin, which has important effects on glucose homeostasis, could reverse hyperglycaemia in *ob/ob* mice before body weight is corrected. Leptin supplementation appears to be an effective therapy, which significantly reduces hepatic steatosis, cell injury, metabolic profile, and transaminase activity in hypoleptinemic lipodystrophic patients with NASH[48]. In our study, similar to adiponectin, the gene and protein levels of leptin in mature 3T3-L1 adipocytes increased significantly after GW4064 treatment. This result may be due to the fact that the FXR agonist promotes the differentiation of adipocytes and is consistent with the positive correlation between leptin levels and fat cell volume[15]. Interestingly, the mRNA expression of OB-Rb in the HepG2 cells increased in a time-dependent manner after GW4064 therapy. Recent studies have demonstrated that the expression of OB-Rb in the liver of NAFLD patients is lower than normal, resulting in leptin resistance[49,50]. In addition, our study showed that OB-Rb had a low basal expression in the 3T3-L1 cells and increased its expression during cell differentiation, which indicates that leptin could act on adipocytes *via* autocrine or paracrine pathways. However, no information is yet available on the effect of GW4064 on the expression of leptin in 3T3-L1 cells.

The third adipokine studied in our study was resistin which is mainly secreted by macrophages, rather than by adipocytes, in humans[51]. Several experiments in rodent models have shown that resistin may induce glucose homeostasis, insulin resistance, and hepatic steatosis[52-54]. However, no human studies have demonstrated a clear relationship between resistin and NAFLD. In our study, GW4064 treatment did not affect resistin expression, so we did not explore further this aspect. Since resistin had no specific receptor, we did not study its receptor.

Peroxisome proliferator activated receptor-gamma (PPAR-γ), which belongs to the nuclear hormone receptor superfamily, is highly expressed in the adipose tissue. PPAR-γ plays a key role in the regulation of adipocyte

differentiation and glucose metabolism[28]. PPAR-γ appears to act as a prominent regulator that can stimulate the whole program of adipogenesis and also as a transcriptional activator of many fat specific genes that are involved in lipid synthesis, growth regulation, insulin signaling, adipokine and associated receptor production[55-57]. Rizzo *et al*[15] and Abdelkarim *et al*[58] have shown that the FXR agonist promotes adipocyte differentiation through a mechanism at least partly involving the induction of PPAR-γ expression. In our study, PPAR-γ expression increased along with the FXR increase after GW4064 treatment. We hypothesized that the effects of the FXR agonist on the adipokines and their receptors in 3T3-L1 cells involved the regulation of PPAR-γ expression. Since the differentiation of fat cells is affected by various other factors, further studies are warranted to elucidate the precise mechanisms whereby FXR agonists are able to impact the adipokines and their receptors.

This study described the expression of adiponectin, leptin, resistin and their receptors during the differentiation of 3T3-L1 adipocytes. We have demonstrated that the FXR agonist, GW4064, increased gene expression and protein levels of adiponectin and leptin, and gene expression of AdipoR2. Moreover, in HepG2 cells, GW4064 enhanced AdipoR2 and OB-Rb gene expression. This study also provides evidence that the FXR agonist regulated adipokines and expression of the associated receptor genes at least partly through the PPAR-γ pathway. Globally, these data may pave the road for a new therapeutic approach in the field of nonalcoholic fatty liver disease. Since adipokines and their receptors also play important roles in other metabolic diseases, FXR agonists might be of interest in therapeutic approaches for other metabolic diseases.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide, affecting 20%-40% of the general population in industrialized countries. However, up to now, the only effective treatment for NAFLD consists of weight loss with lifestyle modifications because no drugs have yet been approved by international agencies. Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily of ligand-activated receptors. Many studies have reported that FXR agonists could prevent and slow down the progress of NAFLD, but the underlying mechanisms remain unclear. Adipokines, secreted by the white adipose tissue, take part in the regulation of insulin resistance, energy metabolism and metabolic syndromes. They are also thought to play an important role in the pathogenesis of NAFLD. Studies have demonstrated that FXR is expressed in the adipose tissue, where its activation promotes the process of adipocyte differentiation. However, the effects of FXR activation on the adipokines and their receptors are not yet fully understood.

Research frontiers

Initially, FXR was thought to have an important role in regulating bile acid metabolism, but increasing data have proved that FXR also plays a key role in the progress of NAFLD. So far, some FXR agonists have been just used in a few clinical trials, but the pharmacology and adverse reactions are still unclear. An improved understanding of mechanisms whereby FXR agonists act on NAFLD is needed.

Innovations and breakthroughs

Both FXR and adipokines play important roles in the progress of NAFLD, and some studies have demonstrated that FXR activation could promote the process of adipocyte differentiation. This study highlights for the first time the effect of GW4064, a synthetic FXR agonist, on adipokines and their receptors. The authors have also proposed a mechanistic basis for using FXR agonists in the treatment of NAFLD. FXR agonists may also be considered for the treatment of other metabolic diseases because adipokines and their receptors are key to those as well.

Applications

The study results strongly suggest that FXR agonist may be a potential therapeutic drug that could be used not only in NAFLD but also in other metabolic diseases.

Peer review

This is a good study in which the authors demonstrate the effect of an FXR agonist on adipokines and their receptors in 3T3-L1 adipocytes and HepG2 cells. The results suggest that the effect of FXR agonist on NAFLD may be partially through regulating the expression of adipokines and their receptors. The study provides more evidence for the use of FXR agonists in the treatment of NAFLD and other metabolic diseases.

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P- Reviewer: Marie JC **S- Editor**: Ding Y **L- Editor**: Wang TQ
E- Editor: Liu XM

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ISSN 1007-9327