Peroxisome Proliferator Activated Receptor Delta Agonist Attenuates Hepatic Steatosis by Anti-inflammatory Mechanism

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Directed by Professor Choon Hee Chung

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Celebrating the day, December 21, 2010

Written by Mi Young Lee

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Peroxisome proliferator receptor delta agonist attenuates hepatic steatosis by anti-inflammatory mechanism

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It is generally known that peroxisome proliferator-activated receptors (PPARs) involved in lipid and carbohydrate metabolism, inflammation, and cell differentiation. Currently, three genes in the PPAR family, PPAR-α, PPAR-γ, and PPAR-δ were discovered. Although PPAR-α (fibrate) and PPAR-γ (thiazolidinediones) have been used as chemical tools to uncover other biological roles for the PPARs, PPAR-δ has not been fully investigated. In this study, we examined the effects of the PPARδ agonist GW0742 on fatty liver changes in a type 2 diabetic rat model and HepG2 cells.

We investigated the effects of PPARδ agonist GW0742 on fatty liver changes in obese diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and nondiabetic control Long-Evans Tokushima Otsuka (LETO) rats. Intraheptic triglyceride contents were investigated in the liver tissue of OLETF rats. Expression of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), monocyte chemo-attractant

protein-1 (MCP-1), and peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α gene were evaluated in OLETF rats and HepG2 cells.

Rats treated with GW0742 (10 mg/kg/day) from 26 to 36 weeks showed low glucose levels, improved insulin sensitivity, and attenuated fatty infiltration of the liver. In liver tissues, mRNA expressions of TNF-α, MCP-1, and PGC-1α were significantly decreased in diabetic rats treated with GW0742 compared to the diabetic control rats. We also observed that GW0742 had inhibitory effects on palmitate induced fatty accumulation and inflammatory markers in HepG2 cells.

The PPARδ agonist (GW0742) may attenuate hepatic fat accumulation by anti-inflammatory mechanism and by reducing hepatic PGC-1α gene expression.

Key words: peroxisome proliferator-activated receptor δ, fatty liver, inflammation

Peroxisome proliferator receptor delta agonist attenuates hepatic steatosis by anti-inflammatory mechanism

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I. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) heterodimerized with the retinoid X-receptor (RXR), one of the nuclear receptors, have been known to be involved in lipid and carbohydrate metabolism after binding to peroxisome proliferators response element (PPRE)[1]. Currently, three genes (PPAR-α, PPAR-γ, and PPAR-δ) in the PPAR family are known. PPAR-α is most abundantly expressed in liver and has the effect of diminishing circulating triglycerides and increasing high dense lipoprotein (HDL) cholesterol. PPAR-γ is present in high concentrations in adipose tissue and improves insulin sensitivity. These effects are well documented, but the function of PPAR-δ has not been fully investigated[2].

Many studies have reported that low-grade chronic systemic inflammatory response to nutrient excess is related to obesity, insulin resistance, type 2 diabetes mellitus, and non-alcoholic fatty liver disease (NAFLD), which refers to a wide spectrum of liver disease ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), and cirrhosis[3-5]. It is reported that hepatic steatosis, inflammation, and apoptosis are induced by neutrophil infiltration and the production of pro-inflammatory cytokines associated with excessive free fatty acid released from adipocytes[6].

Recent studies showed that a PPARδ agonist could prevent lipopolysaccharide (LPS)-induced expression and secretion of pro-inflammatory cytokines in 3T3-L1 adipocytes and reduce atherosclerosis through anti-inflammatory effects in apoE knockout mice[7-8]. However, it is not known that the PPARδ agonist could affect on NAFLD through anti-inflammatory mechanism so far.

In this study, we investigated whether the PPARδ agonist GW0742 could improve fatty liver changes by decreasing inflammatory cytokines in a type 2 diabetic rat model and in the HepG2 cell line. We also examined whether the PPARδ agonist altered expression of genes related to fat accumulation in the liver such as peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α and carnitine palmityl transferase-1 (CPT-1).

II. MATERIALS AND METHODS

2.1. Reagents

The synthetic PPARδ agonist GW0742 was kindly provided by GlaxoSmithKline (Brentford, United Kingdom). Palmitate and other chemicals were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Treatment of rats

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Yonsei University at Wonju Campus. At 25 weeks, 18 male Otsuka-Long-Evans-Tokushima-Fatty (OLETF, Otsuka Pharmaceutical, Tokushima, Japan) and 9 Long-Evans-Tokushima-Otsuka (LETO) rats were divided into three groups: diabetic control group, GW0742 treated diabetic rat group, and normal control group. Animals were housed at constant temperature (20-22ºC) and humidity (50-60%) with a 12-hour light and 12-hour dark cycle. They had free access to water and standard rat chow until 36 weeks of age. The experimental group received GW0742 (10 mg/kg/day) by oral gavage tube from 26 weeks to 36 weeks. At 25 and 35 weeks, body weight, intraperitoneal glucose tolerance test (IPGTT), and intravenous insulin tolerance test (IVITT) were measured and a 24-hour urine sample was collected. Plasma glucose disposal rate (Kitt; %/min), which indicates the time necessary to reduce the basal glucose level by one-half, was calculated as $0.693/t_{1/2}$, where $t_{1/2}$ was determined from the slope of the glycemic concentrations from 3 to 15 minutes after intravenous regular insulin injection (0.1 U/kg). At 36 weeks,

experimental rats were anesthetized with Zoletil® (Virbac Laboratories, Carros, France) by intraperitoneal injection and liver tissues were extracted. A portion of the liver tissue sample was frozen using liquid nitrogen and kept at -70ºC for mRNA and protein analysis. Another portion was fixed in 4% paraformaldehyde for 48 hours and embedded in paraffin for histological examination.

2.3. Cell cultures

3T3-L1 preadipocytes (American Type Culture Collection) were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum. Differentiation was induced 2 days after confluence (day 0) in DMEM containing 10% fetal bovine serum (FBS), 500 mol/L methylisobutylxanthine, 0.25 mol/L dexamethasone, and 10 g/mL insulin for 48 hours. Cells were then incubated in 10% FBS/DMEM with insulin for 8 days. Medium was changed every 2 days. When fat droplets were observed in 90% of cells, adipocytes were incubated for 96 hours with 1 μM/L GW0742 and then 10 ng/mL TNF-α for 48 hours, and nuclear extracts prepared from the adipocytes.

HepG2 cells obtained from Korean Cell Line Bank (KCLB, Seoul, Korea) were cultured in minimum Eagle's medium (Invitrogen Life Technologies Inc., Carlsbad, CA) supplemented with 10% FBS (HyClone Laboratories Inc, Logan, UT), 100 units/mL penicillin (Invitrogen), and 0.1 mg/mL streptomycin (Invitrogen). Cells were maintained at 37°C with humidified air and CO_2 (5%). After 24 hours, indicated concentrations of GW0742 (1 or 10 μM/L) dissolved in dimethylsufoxide (DMSO) (Sigma Aldrich) or DMSO was added to the medium. After 2 hours, palmitate (0.3

mmol/L) was added to the medium and cells were cultured for an additional 24 hours before lysis and purification of total RNA. Subconfluent monolayers of HepG2 cells were stained with Oil-Red-O to determine fat accumulation. Dishes were washed with cold phosphate-buffered saline and fixed in 10% paraformaldehyde for 5 minutes. Oil-Red-O was added with agitation for 15 minutes, followed by washing in 60% isopropanol. Cells were rinsed in distilled water and examined by light microscopy.

2.4. Histologic examination of liver and serum chemistry

Paraffin-embedded 5-μm liver tissue slices were stained with hematoxylin and eosin (H&E). Blood samples were taken by cardiac puncture and serum levels of total cholesterol, triglyceride (TG), HDL cholesterol, aspartate transaminase (AST), and alanine transaminase (ALT) (Fuji DRI Chem 3500, Tokyo, Japan) were measured. Rat ELISA kits for adiponectin (AdipoGen Inc, Seoul, Korea) and monocyte chemo-attractant protein-1 (MCP-1; Invitrogen) were used.

2.5. Intrahepatic triglyceride and free fatty acid

Frozen rat liver tissues were homogenized in 1 mL of deionized water before adding 5 mL of chloroform and methanol, and centrifuging at 4ºC and 35,000 rpm for 10 min. The lower phase was transferred to a clean tube and 250 μL of 1% Triton X-100 in chloroform was added. After vacuum drying to remove the chloroform, triglyceride and free fatty acid levels were measured using a kit (Cayman Chemical Company, Ann Arbor, MI, USA).

2.6. Real time RT-PCR

Total RNA was isolated from 3T3-L1 adipocytes, HepG2 cells and liver tissues using TRIzol reagent (Invitrogen) as described by the manufacturer. Complementary DNA (cDNA) was synthesized from 1 μg of RNA using a M-MLV reverse transcription system (Promega, Madison, WI). Real time RT-PCR was performed using a SYBR Green RT-PCR kit (Qiagen, Valencia, CA) and measured with a Roter-Gene RG-3000 cycler (Corbett Research, Mortlake, Australia). Relative gene expression for all analyzed genes was by the $2^{-\Delta\Delta Ct}$ method, normalizing to GAPDH gene expression. Primer pairs are in Table 1.

Table 1. Primers for real time RT-PCR

Gene name		Primer sequence
3T3-L1 adipocyte		
MCP-1	Forward	5’- CTTGCCTAATCCACAGACTG-3’
	Reverse	5’-GCCTGAACAGCACCACTA-3’
GAPDH	Forward	5’-CTGGAGAAACCTGCCAAGTA-3’
	Reverse	5’-AGTGGGAGTTGCTGTTGAAG-3’
HepG2 cell		
TNF-α	Forward	5’- CCAGACCAAGGTCAACCTC-3’
	Reverse	5’-CCAGATAGATGGGCTCATACC-3’
IL-6	Forward	5’-AAAAGTCCTGATCCAGTTC-3’
	Reverse	5’-GAGATGAGTTGTCATGTCC-3’
PGC-1α	Forward	5’-TGTGCAACTCTCTGGAACTG-3’
	Reverse	5’-TGAGGACTTGCTGAGTGGTG-3’
NF-kB	Forward	5’-GAAGAAAATGGTGGAGTCTG-3’
	Reverse	5’-GGTTCACTAGTTTCCAAGTC-3’
GAPDH	Forward	5’-ACCCACTCCTCCACCTTTG-3’
	Reverse	5’-CTCTTGTGCTCTTGCTGGG-3’
OLETF rat liver tissue		
MCP-1	Forward	5‘-TCTCTTCCTCCACCACTATGCA-3'
	Reverse	5‘-GGCTGAGACAGCACGTGGAT-3'
TNF-α	Forward	5'-ATGGATCTCAAAGACAACCA-3'
	Reverse	5'-TCCTGGTATGAAATGGCAAA-3’
PGC-1α	Forward	5'-TGTTCGATGTGTCGCCTTCT -3’
	Reverse	5'-GAACGAGAGCGCATCCTTTG -3’
CPT1	Forward	5'-GCTCGCACATTACAAGGACAT -3‘
	Reverse	5'-TGGACACCACATAGAGGCAG -3’

2.7. Statistical analyses

All data are presented as means ± S.D. Statistical analysis was carried out by ANOVA, followed by Tukey test for multiple comparisons using the SPSS 17.0 program. Differences were considered significant at $p< 0.05$.

III. RESULTS

3.1. Anthropometric and biochemical characteristics of experimental rats

Compared to the control LETO group, the body weight of the diabetic control (DM) and GW0742-treated diabetic (DM+GW) groups was significantly increased over the experimental period. Although no difference in body weight was observed between the diabetic control and GW0742 treated diabetic rat group at the end of the study, the amount gained in body weight was significantly lower in the GW0742 treated diabetic rat group compared to the diabetic control group. Epididymal fat weight on the left and right sides of the control group were significantly lower than the diabetic rat groups. Epididymal fat weights of the GW0742 treated diabetic rat group were significantly lower than the diabetic control group. The total cholesterol level was significantly increased in only the diabetic control group compared to the normal control group. However, triglyceride levels were significantly increased in both the diabetic control and GW0742 treated diabetic rat groups compared to the normal control group. No differences were observed in the serum levels of adiponectin or MCP-1 among the groups (Table 2).

Table 2. Anthropometric and biochemical characteristics of experimental rats

	Control	DM	DM+GW
Initial body weights (g)	482.00 ± 31.80	562.44 ± 32.84*	589.00 ± 49.7*
Final body weights (g)	518.11 ± 35.83	627.00 ± 9.02*	616.37 ± 58.57*
Weight change	36.11 ± 10.50†	64.55 ± 24.30*	26.62 ± 25.02†
Epididymal fat pad, left (g)	5.11 ± 0.96	8.66 ± 0.75*	7.22 ± 1.20*,†
Epididymal fat pad, right (g)	5.05 ± 1.37	8.22 ± 0.87*	6.44 ± 1.44*,†
Total cholesterol (mg/dL)	70.33 ± 8.86	92.22 ± 22.40*	86.33 ± 27.52
Triglyceride (mg/dL)	23.33 ± 10.36	189.22 ± 86.81*	225.88 ± 132.60*
HDL cholesterol (mg/dL)	58.11 ± 6.03	67.66 ± 13.66	63.44 ± 15.58
AST (IU/L)	56.55 ± 6.30	50.11 ± 15.71	47.55 ± 13.48
ALT (IU/L)	25.00 ± 2.95	28.66 ± 6.87	39.55 ± 24.54*
Adiponectin (μg/mL)	5.29 ± 0.43	5.6 ± 0.90	5.39 ± 0.97
MCP-1 (pg/mL)	82.19 ± 32.25	91.74 ± 34.11	90.68 ± 24.60

Data are mean ± S.D.; Control, normal control group; DM, diabetic control group; DM+GW, GW0742 treated diabetic rat group; HDL, high density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; MCP-1, monocyte chemoattractant protein-1; *, $p < 0.05$ vs. Control; †, $p < 0.05$ vs. DM.

3.2. Anti-inflammatory effects of GW0742 in 3T3-L1 adipocytes

In 3T3-L1 adipocytes, the PPARδ agonist GW0742 at 1 μM/L significantly inhibited TNF-α-induced MCP-1 mRNA expression. (Fig. 1).

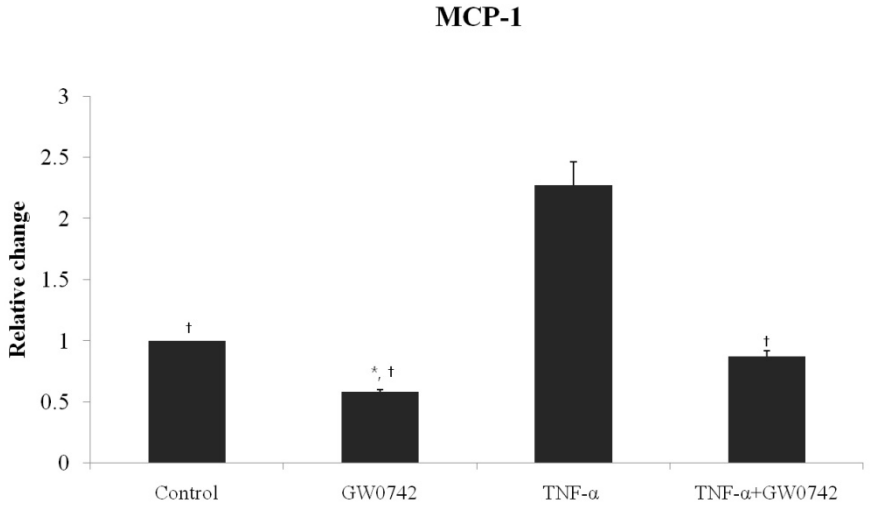

Figure 1. Effects of the PPARδ agonist GW0742 on MCP-1 mRNA expression induced by TNF-α. TNF-α induced MCP-1 mRNA expression was significantly inhibited by 1 μM/L concentration of GW0742 in 3T3-L1 adipocytes. PPARδ, peroxisome proliferator receptor δ; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1. *, $p < 0.05$ vs Control; †, $p < 0.05$ vs TNF-α.

3.3. Intraperitoneal glucose tolerance test and intravenous insulin tolerance test

Changes in plasma glucose during IPGTT in each experimental group at 35 weeks of age are illustrated in Figure 2A. Plasma glucose level at each time point of 15, 30, 60, 90, and 120 minutes was significantly decreased in the GW0742 treated diabetic group compared to the diabetic control group. Plasma glucose levels during IVITT in each experimental group are shown in Figure 2B and measured Kitt value was shown in Table 3. The plasma glucose level of diabetic control group was significantly increased during IVITT periods compared to the normal control group and the glucose level of the GW0742 treated diabetic rat group was not different to those of normal control group. The Kitt value of the GW0742 treated diabetic rat group was significantly decreased compared to the diabetic control group.

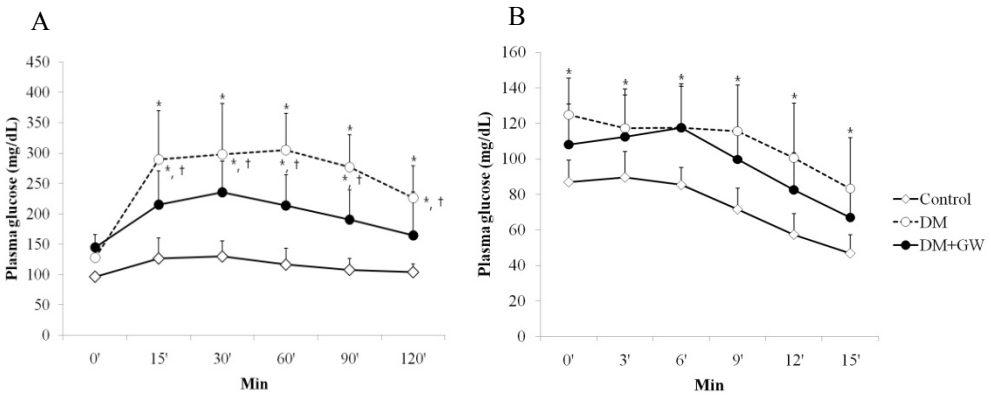

Figure 2. Changes in plasma glucose during IPGTT and IVITT. Plasma glucose responses in the IPGTT (A) and IVITT (B) in the normal control group (Control, n=9), diabetic control group (DM, n=9), and GW0742 treated diabetic rat group (DM+GW, n=9) at the end of the study. *, $p < 0.05$ vs Control; †, $p < 0.05$ vs DM.

Table 3. Kitt value calculated from glucose level during IVGTT

	Kitt
Control	4.13 ± 0.83
DM	1.87 ± 1.45*
DM+GW	3.78 ± 1.31*, †

Values are presented as mean ± S.D. Kitt, rate constant for glucose disappearance in the insulin tolerance test. Control, normal control group; DM, diabetic control group; DM+GW, GW0742 treated diabetic rat group *, $p < 0.05$ vs Control; †, $p < 0.05$ vs DM.

3.4. Histological finding and intrahepatic lipid levels of liver

All liver tissues were analyzed by H&E staining (Fig. 3). Higher fatty droplet accumulation was observed in diabetic rat groups compared to the normal control group. However, GW0742 treated diabetic rat group showed decreased fatty accumulation compared to the diabetic control group. The levels of TG and FFA from liver tissues in diabetic control rat group were significantly higher than the normal control group. However, compared to the diabetic control group, the GW0742 treated diabetic rat group had significantly lower intrahepatic TG levels and slightly decreased intrahepatic FFA levels, although this was not significant (Fig. 4).

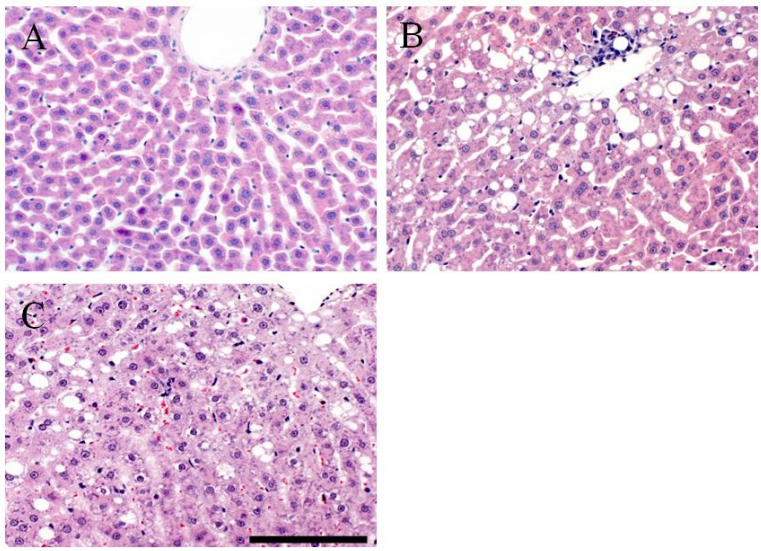

Figure 3. Histological findings of liver. Representative images of H&E staining in the normal control group (A), the diabetic control group (B), and the GW0742 treated diabetic rat group (C) were shown. In the diabetic control group, macrovesicular steatosis occupied greater than normal control group. GW0742 treated diabetic rat group showed markedly decreased fatty accumulation compared to the diabetic control group. ×200; scale bar, 50 μm.

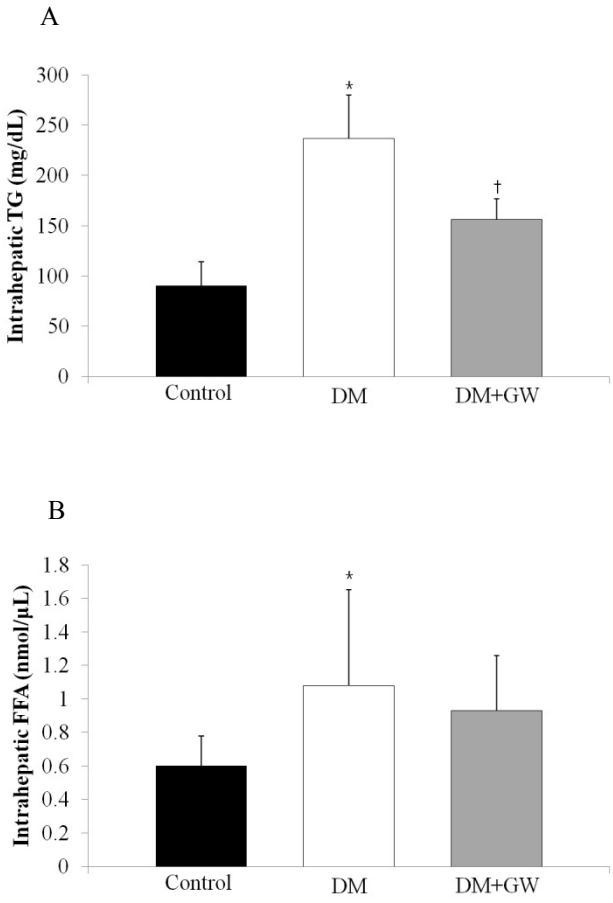

Figure 4. Analysis of intrahepatic TG (A) and FFA (B) levels. The increased TG level of the diabetic control group was significantly decreased by GW0742 treatment. The intrahepatic FFA level of the GW0742 treated rat group showed a tendency to decrease. TG, triglyceride; FFA, free fatty acid; Control, normal control group; DM, diabetic control group; DM+GW, GW0742 treated diabetic rat group. *, $p < 0.05$ vs Control; †, $p < 0.05$ vs DM.

3.5. Real time RT-PCR for TNF-α, MCP-1, PGC-1α, and CPT-1 in rat liver tissues

To determine the anti-inflammatory effects of GW0742 in the liver, we used real-time RT-PCR to examine TNF-α and MCP-1 mRNA in experimental rat liver tissues, as representative inflammatory cytokines. Quantitative analysis revealed that TNF-α and MCP-1 mRNA levels were significantly increased in the diabetic control group compared to the normal control group, and the GW0742 treated diabetic rat group showed significantly decreased TNF-α and MCP-1 mRNA compared to the diabetic control group (Fig. 5A and 5B). To investigate whether GW0742 altered gene expression related to fat accumulation in liver, we examined the mRNA levels of PGC-1α, and CPT-1. The PGC-1α mRNA in the diabetic control group showed an approximately three-fold increase compared to the normal control group and PGC-1α mRNA was significantly decreased in the GW0742 treated diabetic rat group (Fig. 5C). In contrast, the mRNA of CPT-1 was significantly decreased in the diabetic control and GW0742 treated diabetic rat groups compared to the normal control group. However, no difference in CPT-1 mRNA levels was observed between the diabetic control and GW0742 treated diabetic rat groups (Fig. 5D).

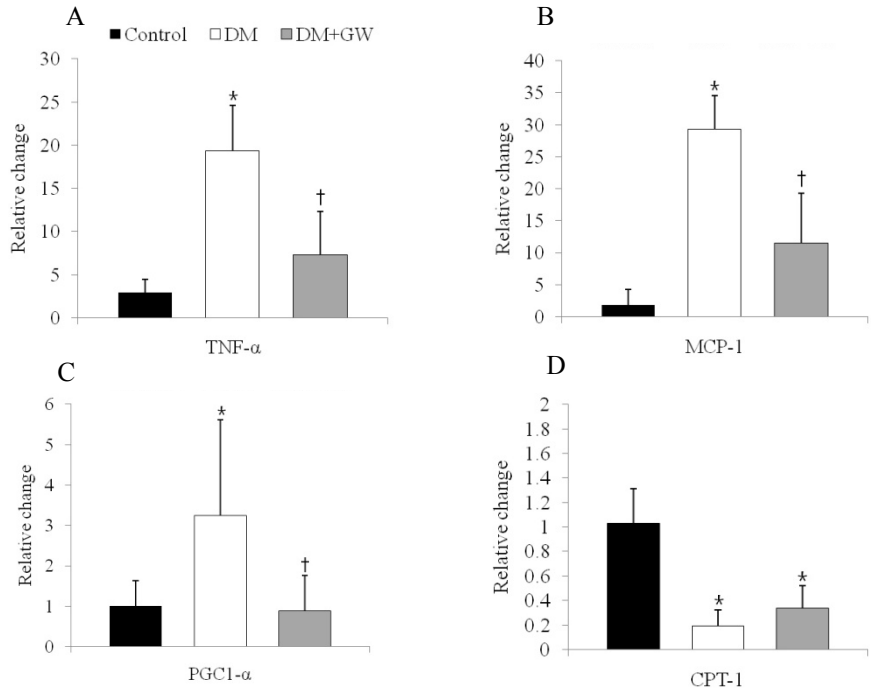

Figure 5. Relative mRNA expression of TNF-α (A), MCP-1 (B), PGC-1α (C), and CPT-1 (D) in rat liver tissues. Inflammatory cytokine expression was significantly decreased in GW0742 treated diabetic rat group (DM+GW) compared to the diabetic control group (DM). The mRNA expression of PGC-1α was significantly decreased by GW0742 treatment. The mRNA expression of CPT-1 was significantly decreased in both DM and DM+GW groups compared with that of normal control group. *, $p < 0.05$ vs Control; †, $p < 0.05$ vs DM.

3.6. Oil-Red-O stain of HepG2 cells

Human HepG2 cells were used to extend the results from OLETF rats to a cellular model. To induce lipid accumulation in HepG2 cells, cells were exposed to a pathophysiologically relevant concentration of the plasma free fatty acid palmitate and various concentrations of GW0742. Intracellular lipid vacuoles induced by palmitate were confirmed by Oil-red-O staining (Fig. 6). The increase in lipid vacuoles in palmitate-treated HepG2 cells was markedly diminished by co-administration of 1 or 10 μM/L of GW0742 (Fig. 6). The expression levels of TNF-α, interleukin-6 (IL-6), nuclear factor-κB (NF-κB) and PGC-1α were slightly increased in palmitate-treated HepG2 cells compared to the control group. This increase in mRNA levels was slightly decreased by GW0742 at different concentrations, but these differences were not significant (Fig. 7).

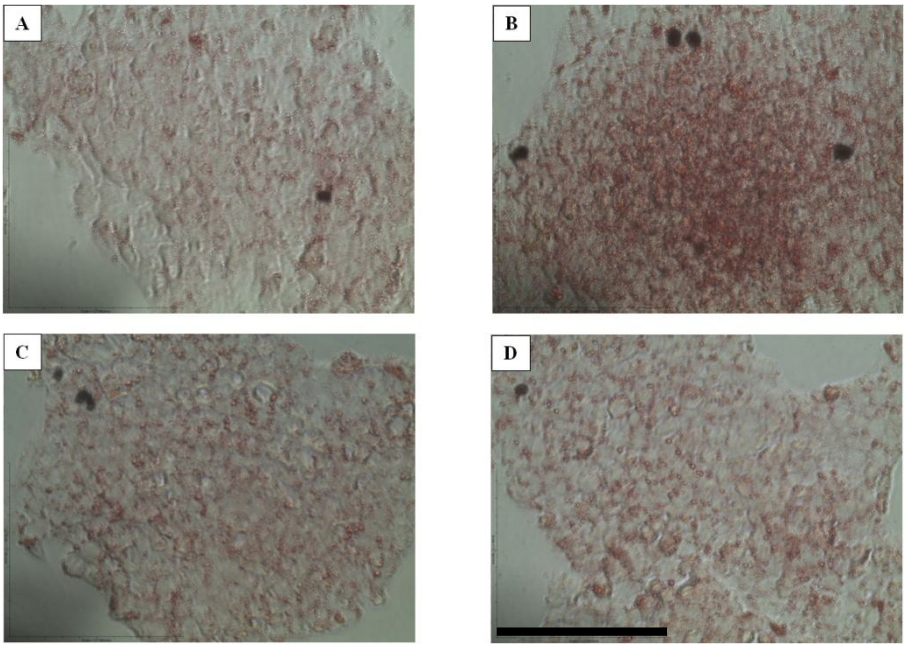

Figure 6. Treatment with GW0742 ameliorates intracellular lipid accumulation in HepG2 cells. HepG2 cells were cultured with DMSO control (A), 0.3 mM palmitate (B), or 0.3 mM palmitate and 1 μM (C) or 10 μM (D) of GW0742. Cells were stained with Oil-red-O to measure intracellular lipid accumulation. Intracellular lipid vacuoles were markedly reduced by GW0742 treatment. × 400; scale bar, 127 μm.

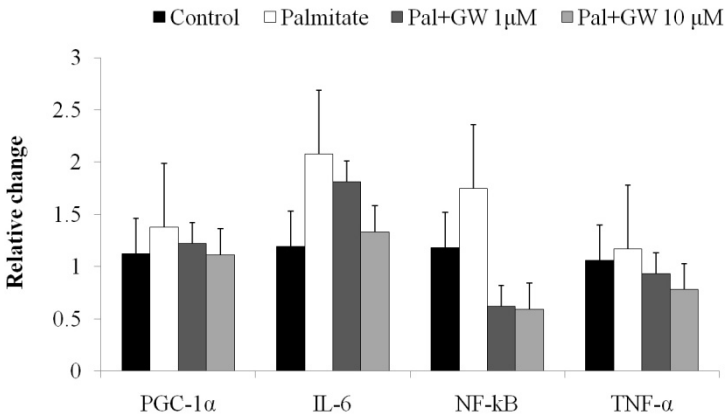

Figure 7. The mRNA expression levels of TNF-α, IL-6, NF-κB and PGC-1α in HepG2 cells. Cells treated with GW0742 showed a tendency to have decreased mRNA for TNF-α, IL-6, NF-κB and PGC-1α. TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; NF-κB, nuclear factor-κB; PGC-1α, peroxisome proliferator activated receptor (PPAR)-γ coactivator-1α.

IV. DISCUSSION

In this study, we found a protective function for the PPARδ agonist GW0742 on hepatic steatosis. This protective role was ascertained in both a type 2 diabetic rat model and in HepG2 cells.

Among the PPARs subtypes, PPAR-γ and PPAR-α are already targets for the treatment of type 2 diabetes and dyslipidemia[9-11]. On the other hand, the therapeutic potential of PPAR-δ on insulin resistance and fatty acid metabolism is still under investigation. The known key features of PPAR-δ activation are an increase in fatty acid oxidation in skeletal muscle and cholesterol flux from peripheral tissues[12-14]. Recently, a PPAR-δ agonist was reported to reduce inflammation in adipose tissue or vascular smooth muscle cells[7-8]. Because inflammatory process was known to participate in insulin resistance, type 2 diabetes, and cardiovascular disease[3-4], blocking inflammation might be one of the targets of intervention in insulin resistance. Recent studies demonstrated that PPARδ agonist could inhibit lipopolysaccharide (LPS) induced expression of MCP-1 and IL-6 in 3T3-L1 adipocytes and TNF-α induced NF-κB activation in human umbilical vein endothelial cells (HUVECs)[8, 15]. To confirm the anti-inflammatory effect of PPARδ agonist, we investigated MCP-1 expression level, which is increased in inflammatory and insulin resistant states[16], in 3T3-L1 adipocytes. We observed that TNF-α induced MCP-1 expression and a PPARδ agonist significantly suppressed MCP-1 expression in 3T3-L1 adipocytes. This finding is consistent with previous studies that reported reduced cytokine expression levels in the presence of a PPARδ agonist. In addition, we found that PPARδ agonist treatment could decrease plasma glucose levels and improve insulin

sensitivity marker, Kitt. Although PPARδ agonist treatment did not cause differences in the body weights of OLETF rats, the weight gain of PPARδ agonist-treated OLETF rats was significantly lower than the diabetic control group, and epididymal fat weights were significantly decreased in PPARδ agonist-treated OLETF rats. These findings indirectly implicated the PPAR δ agonist in effects on insulin resistance.

In patients with metabolic syndrome, the prevalence of non-alcoholic fatty liver disease (NAFLD) is two- or three times higher than in general population[17]. Currently, there is no approved therapy and identifying research area for the effective treatment of NAFLD. NAFLD refers to a wide spectrum of liver disease ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH) and cirrhosis. The pathologic findings of NASH are characterized by microvesicular or macrovesicular steatosis, inflammation, hepatocyte degeneration, and sometimes fibrosis[18]. In particular, inflammatory processes secondary to insulin resistance are regarded as a characteristic finding of NASH[19]. To investigate the effects of PPARδ agonist on hepatic steatosis, we performed experiments in a type 2 diabetic rat model and in HepG2 cells. Significant differences in liver histology were seen between diabetic control rats and PPARδ agonist treated rats. In addition, lipid vacuoles were significantly decreased in HepG2 cells cultured with palmitate and a PPARδ agonist compared to cells treated with palmitate only. Because palmitate is known to induce a hyperlipidemic condition via inflammatory liver injury[20-21], it could be thought that palmitate treatment sufficiently caused hepatic steatosis and inflammation. These findings may result from a reduction in liver tissue inflammation, as confirmed by RT-PCR. The anti-inflammatory effects of the PPARδ agonist on the liver were

surprising, and could be of clinical interest because few effective treatment strategies exist for NAFLD. Although the mechanism for the beneficial effect of PPARδ on the liver is partially explained by similar anti-inflammatory activity in adipose tissues and vascular smooth muscle cells, the therapeutic effects on NAFLD could in part be mediated through expression of other hepatic genes. Therefore, we examined genes known to cause fatty liver. By examining various genes, we found that the PPARδ agonist decreased PGC-1α gene expression. PGC-1α is a transcriptional co-activator involved in thermogenesis and energy metabolism[22]. It is well elucidated that PGC-1α could promote fatty acid oxidation, reduce fat accumulation, increase GLUT4 expression in muscle, and eventually lead to improvement of insulin resistance[23-24]. However, PGC-1α expression is reported to increase in type 2 diabetic mice, and increased hepatic PGC-1α expression was reported to increase PEPCK and contribute to fatty liver[25]. Thus, the regulation of hepatic PGC-1α expression is considered another process in fatty liver changes along with inflammation. In our study, PPARδ agonist treatment significantly reduced PGC-1α expression in liver tissue. Also, although the current in vitro results may not show significant differences, potential evidence was seen for reduction of PGC-1α. However, the paradoxical actions of PGC-1α in muscle and liver are controversial, so the effects of the PPARδ agonist on hepatic PGC-1α expression requires further study. In addition, examining the effects of the PPARδ agonist on different tissues would be beneficial.

Patients with type 2 diabetes have a higher risk for NAFLD and other inflammatory processes. According to our results, a PPARδ agonist could function in

glycemic control and affect fatty liver changes in type 2 diabetes and insulin resistance.

V. CONCLUSION

In summary, the PPARδ agonist GW0742 attenuated hyperglycemia and fatty accumulation in liver. These changes were probably due to suppression of inflammatory cytokines such as TNF-α and MCP-1. The beneficial effect on hepatic fatty accumulation was also partially explained by reduction in PGC-1α expression. Our findings suggest that PPARδ agonist has beneficial effects against NAFLD by anti-inflammatory effects and reduction of PGC-1 α.

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ABSTRACT IN KOREAN

PPAR delta 작용제의 항염증 기전에 의한 지방간의 개선 효과

퍼록시좀 증식활성 수용체들은 지질이나 당대사, 염증 반응, 세포분화 등에 관여한다고 알려져 있다. 최근까지 퍼록시좀 증식활성 수용체 알파 (PPAR-α), 퍼록시좀 증식활성 수용체 감마 (PPAR-γ), 그리고 퍼록시좀 증식활성 수용체 델타 (PPAR-δ)의 세가지 형태의 수용체가 발견되었으며 PPAR-α 는 피브릭산 유도체, PPAR-γ 는 치아졸리딘디온계 당뇨병 치료제로 사용되고 있는 반면 PPAR-δ 는 아직 효과가 입증되지 않은 상태이다. 이에 본 연구에서는 PPAR-δ 작용제가 제 2 형 당뇨병 쥐 모델이나 HepG2 세포에서 지방간에 대해 영향을 미치는지 알아보고자 하였다.

지방간에 대한 효과를 보기 위하여 제 2 형 당뇨병 쥐 모델인 Otsuka Long-Evans Tokushima Fatty (OLETF) 쥐에 PPAR-δ 수용체 작용제인 GW0742 를 처리하였다. 쥐의 간조직 내의 지방 농도를 측정하였고 tumor necrosis factor-α (TNF-α)나 monocyte chemo-attractant protein-1 (MCP-1) 등의 염증반응 표지자 및 peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α 발현 정도를 쥐의 지방 조직 및 HepG2 세포에서 GW0742 를 처리한 후 분석하였다.

생후 26 주에서 36 주까지 GW0742 (10 mg/kg/day)를 처리한 쥐 군에서 혈당이 감소하였고 인슐린 저항성이 개선되었으며 지방간이 호전되었다. 간 조직내 TNF- α, MCP-1, 그리고 PGC-1α 의 발현이 GW0742 를 처리한 당뇨병 쥐 군에서 당뇨병 대조군에 비하여 의미 있게 감소하였다. 또한 palmiate 로 유도된 HepG2 세포에서의 지방 침착이 GW0742 처리한 세포군에서 감소하였고 염증성 반응 표지자들이 감소하는 경향을 보였다.

이러한 결과로 PPAR-δ 작용제인 GW0742 가 항염증 기전 및 간조직내 PGC-1α 발현을 억제하여 지방간을 호전시키는 것으로 생각된다.