The effect of rosiglitazone on hepatic LRP1 expression: a novel mechanism of the improvement of atherogenic dyslipidemia by thiazolidinedione drugs in diabetes mellitus

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Directed by Professor Bong Soo Cha

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ABSTRACT

The effect of rosiglitazone on hepatic LRP1 expression: a novel mechanism of the improvement of atherogenic dyslipidemia by thiazolidinedione drugs in diabetes mellitus

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Hepatic low-density lipoprotein receptor-related protein 1 (LRP1) plays an important role in the clearance of circulating remnant lipoproteins. In this study, the effect of thiazolidinedione, an insulin sensitizing drug on the expression and function of hepatic LRP1 was investigated. This study may present a novel mechanism of the improvement of atherogenic dyslipidemia by a thiazolidinedione drug in diabetic patients.

For *in vitro* evaluation, HepG2 cells were treated with various concentrations of rosiglitazone. For *in vivo* study, male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats were used as a diabetic animal model and its normal counterpart respectively. Rats were treated with rosiglitazone for 5 weeks. The expression and function of LRP1 in HepG2 cells and liver samples of rats were analyzed.

LRP1 mRNA and protein expressions were increased by 0.5 and 5 μM of rosiglitazone in HepG2 cells. However, the expression of LRP1 did not change compared to that in non-treated cells at concentrations above 50 μM of rosiglitazone. The electrophoretic mobility shift assay and the reporter assay

showed that rosiglitazone increased the transcriptional activity of the LRP1 promoter by binding peroxisome proliferator-activated receptor-γ to a perxisome proliferator response element of the LRP1 promoter in HepG2 cells. The rosiglitazone-induced up-regulation of the LRP1 promoter activity showed the same tendency with the LRP1 expression. The uptake of ApoE through LRP1 in HepG2 cells was also increased by rosiglitazone. The serum triglyceride level was increased in OLETF rats compared to that in LETO rats and partially recovered with rosiglitazone treatment. Hepatic LRP1 was reduced in OLETF rats compared to that in LETO rats and rosiglitazone treatment increased the hepatic LRP1 in OLETF rats. A high-glucose condition (25 mM of glucose in culture media) reduced the expression of LRP1 in HepG2 cells, and this reduced LRP1 expression was recovered with rosiglitazone.

In conclusion, our data suggest that decreased hepatic LRP1 in a diabetic condition is associated with the development of atherogenic dyslipidemia and that increased hepatic LRP1 by thiazolidinediones contributes to an improvement in atherogenic lipid profiles in diabetic patients.

Key words :

LRP1, diabetes mellitus, thiazolidinedione, atherogenic dyslipidemia, PPARγ

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I. INTRODUCTION

Diabetes mellitus (DM) is a one of major risk factor for cardiovascular disease. Diabetic condition can cause atherogenic dyslipidemia including elevated triglycerides and low high-density lipoprotein-cholesterol (HDL-c)[1, 2]. Increased free fatty acid due to insulin resistance in adipose tissue promotes the hepatic production of triglycerides which are packaged in apolipoprotein B-containing very low-density lipoproteins (VLDLs)[3]. Increased triglyceride-rich lipoproteins, such as chylomicron and VLDL are hydrolyzed to remnant particles[4]. Recent studies have focused on these remnant lipoproteins as atherogenic particles, showing that remnant lipoproteins can penetrate the endothelial wall and remain in the subendothelial space[5]. Remnant lipoproteins have also been reported to be associated with inflammation, endothelial dysfunction, foam cell formation and vascular smooth muscle cell proliferation[5]. Several studies have reported that the level of remnant-like particle-cholesterol

(RLP-c) is increased in diabetic patients[6-8] and is associated with an increased risk of cardiovascular disease[9, 10].

Low-density lipoprotein receptor-related protein 1 (LRP1) is a member of the Low-density lipoprotein receptor gene family, and is a multifunctional scavenger and signaling receptor that binds and internalizes diverse ligands[11]. This cell surface glycoprotein binds apolipoprotein E (ApoE) and serves as a receptor for remnant lipoproteins such as chylomicron remnant and VLDL remnant in the liver[11]. Furthermore, this receptor plays an important role in the clearance of remnant lipoproteins[12-14]. A thiazolidinedione drug, such as rosiglitazone or pioglitazone is an oral hypoglycemic agent that improves insulin resistance by activating the peroxisome proliferator-activated receptor-γ (PPARγ). This agent has also been known to modify the atherogenic lipid profile. In clinical studies, pioglitazone decreased the serum level of triglycerides and RLP-c and increased serum HDL-c[15-18]. Rosiglitazone also decreased serum triglycerides in some animal and clinical studies[19-22]. Pioglitazone has been reported to reduce serum triglycerides by increasing their clearance from the circulation, most likely through increased lipoprotein lipase-mediated lipolysis of VLDLs[23]. One *in vitro* study reported that LRP1 is regulated by PPARγ and that rosiglitazone induced the LRP1 expression in human adipocytes[24]. On the basis of these data, it was hypothesized that thiazolidinediones may increase hepatic LRP1 and the hepatic clearance of remnant lipoproteins. In this study, the effect of rosiglitazone on the expression and function of hepatic LRP1 was investigated. This study may present a novel mechanism of the improvement of atherogenic dyslipidemia by a thiazolidinedione drug in diabetic patients.

II. MATERIALS AND METHODS

1. Cell culture and preparation

The HepG2 cells were cultured in MEM containing 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 mg/ml) at 5% CO_2/95% air and 37 ℃. Rosiglitazone was provided by GlaxoSmithKline (Brentford, Middlesex, UK) and prepared in dimethylsulfoxide (DMSO) at −20°C. HepG2 cells were incubated with the indicated concentrations of rosiglitazone for 48 h by adding the stock solution to the culture media. The final concentration of DMSO in the culture media was adjusted to 0.5% (vol/vol).

2. Animals, diet and treatment

Laboratory animals for all experiments were cared for in accordance with the National Institute of Health's guidelines. The animals were maintained according to the ethical guidelines of our institution, and the experimental protocol was approved by the Committee on Animal Investigations of Yonsei University. Male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, aged four weeks, were supplied by the Tokushima Research Institute, Otsuka Pharmaceuticals (Tokushima, Japan). The rats were housed in a temperature-controlled environment under a 12-hr light/dark cycle and allowed as libitum access to standard chow and water. Male Long-Evans Tokushima Otsuka (LETO) rats (Tokushima Research Institute, Otsuka Pharmaceuticals) were used as non-diabetic counterparts.

All rats were fed standard chow until the age of 20 weeks. Thereafter, OLETF rats were fed a high-fat diet (40% lard, Wellga Inc., Seoul, Korea). OLETF rats were randomly divided into two groups: the vehicle (normal saline)-treated group (n=9) and the rosiglitazone-treated group (n=8). The rosiglitazone-treated rats were administered 4 mg/kg/day of compound in saline via oral gavage using 20 G feeding needles. LETO rats received standard chow

without any drug (n=8). All the animals were treated for five weeks. One day after the OGTT at the age of 25 weeks, all the rats were sacrificed; the livers were extracted, processed, and embedded in paraffin for histological analysis. The remaining tissues were flash-frozen in liquid nitrogen and stored at -80℃ until analysis. Blood was collected by cardiac puncture and stored at -20℃ for the biochemical tests.

3. Oral glucose tolerance test

All the rats underwent an OGTT after an overnight fast using 20% glucose solution (2 g/kg). Blood samples were obtained by tail snipping, and blood glucose levels were measured with a glucose analyzer (Accu-Check; Roche Diagnostics, Basel, Switzerland). Glucose levels were recorded at 0, 15, 30, 60 and 120 min after glucose administration.

4. Biochemical analysis

Blood samples were obtained from the heart at the time of sacrifice and were immediately centrifuged at 5000xg for 5 min. Total cholesterol and triglycerides were determined using an ADVIA 1650 (Bayer, West Haven, CT, USA).

5. Total RNA and cDNA preparation

Total RNA was isolated from HepG2 cells and the rat liver tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and was quantified using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Rockford, IL, USA). Following the RNA extraction, 4 μg of RNA were treated with 1 U RNase-free DNase I to remove all contaminating genomic DNA. After removing the DNase I, DNase-treated RNA was subsequently used for cDNA synthesis using MMLV reverse transcriptase (Promega, Madison, WI, USA)

according to the manufacturer's protocol. The synthesized cDNA was stored at -20℃ for later use.

6. Quantitative real-time PCR (RT-PCR)

Quantitative RT-PCR analysis was performed using Taqman assay kits for LRP1 (Hs00233856_m1, R701503964 g1) with the ABI 7500 instrument (Applied Biosystems, Foster City, CA). The β-actin (Hs99999903_m1, R700667669_m1) gene was used as an invariant control. PCR reactions were carried out in triplicate reactions in a final volume of 20 μl according to the manufacturer's protocol. For each assay, a standard curve was obtained by analyzing a series of dilutions of pooled cDNA samples for the relevant gene. Data were analyzed with Sequence Detector 1.7 software (Applied Biosystems). β-actin was used as an invariant control and the results were expressed as a ratio of the gene expression relative to that of β-actin.

7. Immunoblot analysis

Cell lysates were prepared using MPER® (Thermo Scientific) and aliquots of cell lysates and tissue homogenateswere denatured under reducing conditions (1.75% SDS, 15 mM 2-mercaptoethanol) for 5 min at 100℃. The total protein amount in each cell lysate was determined by Bradford assay (Sigma-Aldrich, St Louis, MO, USA). Cell lysates including 10 μg of protein were loaded to SDS-PAGE for immunoblot analysis. For LRP1, nitrocellulose membranes were incubated with anti-LRP1 antibody (Epitomics, Burlingame, CA, USA) at a 1:1,500 dilution overnight at 4℃, then subsequently with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1:5,000 dilution for 1 h at room temperature. The signals were detected with the ECL Western Blotting Analysis System (Thermo Scientific). β-actin immunoreactivity which detected with monoclonal anti-β-actin antibody (Sigma-Aldrich) at a 1:5,000 dilution and

horseradish peroxidase-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology) at an 1:5,000 dilution, was used as a loading control.

8. Electrophoretic mobility shift assay (EMSA)

The nuclear protein extracts of HepG2 cells were prepared using the Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific). Double-stranded oligonucletides of a perxisome proliferator response element (PPRE) in human *LRP1* promoter (5′-CCCCGCTCCTTGAACTCTGACATGCAGACACCTA-3′) were synthesized and end-labeled with biotin[24]. Also, the oligonucleotides of the mutated form of the *LRP1* PPRE (5′-CCCCGCTCCTTGAACTCAACGATGCAGACACCTA-3′) were synthesized and biotin-labeled[24]. Nuclear protein extracts were incubated with the labeled oligonucleotides in the presence of poly(dI-dC) in a binding buffer containing 10mM Tris-HCl, 5 mM MgCl2, 0.5mM dithiothreitol, 5 mM EDTA, 5% glycerol and 50mM KCl at room temperature for 20 min. DNA-protein complexes were then resolved by electrophoresis on 10% polyacrylamide gels and visualized by the Chemiluminescent Nucleic Acid Detection Module (Thermo Scientific).

For the supershift assays, co-incubation was performed using 0.5 μg of anti-PPARγ antibody (R&D systems, Minneapolis, MN, USA) before adding the labeled oligonucleotides. The reaction was carried out at 4 ℃ for 30 min. To examine the specificity, a 200-fold excess of unlabeled double-stranded oligonucleotides was added as a competitor in the binding reaction.

9. Construction of the LRP1 promoter-reporter vector

The *LRP1* promoter-reporter was constructed according to the method previously reported by Gauthier *et al.*[24]. The promoter region spanning the upstream 1.9 kb of the 5'-flanking region of *LRP1* which contains a PPRE was

amplified by PCR using the LRP1-BAC construct as a template (Source BioScience imaGenes, Berlin, Germany) using the following primers: 5′-GCAACGAGCTCCGTAAAAGGGGGAAG-3′ and 5′-GCAGCAGATCTTTCCCCGGACTGAAG-3′. The amplified fragment was subcloned into the *Sac*I and *Bgl*II sites of the firefly luciferase reporter vector, pGL3-Basic (Promega), and designated as pGL3-PPRE. The integrity of the reporter plasmid sequences was confirmed by DNA sequencing.

10. Transient transfection assays

HepG2 cells were seeded at a density of 1.5 x 10^5 cells/well in 6-well plates 48 h prior to transfection. When the cell density reached a confluency of 90%, cells were co-transfected with 4 μg of pGL3-PPRE and 0.25 μg of pRL-CMV, the Renilla luciferase reporter vector using Lipofectamin 2000® (Invitrogen) acoording to the manufacturer's protocol. The pGL3-Basic vector was used for the negative control. Four hours after the transfection, the cells were treated with the indicated concentrations of rosiglitazone for 48 h. The cell lysates were prepared with 250 μl of reporter lysis buffer (Promega).

Luciferase activities derived from both firefly (pGL3-PPRE) and Renilla (pRL-CMV) proteins were measured using the dual luciferase reporter assay system (Promega) using a Berthold luminometer (Berthold, Wildbad, Germany).The firefly lucifrease activity was normalized with Renilla luciferase activity to minimize any experimental variability caused by differences in cell viability or transfection efficiency.

11. Apo E uptake analysis

HepG2 cells were treated with the indicated doncentrations of rosiglitazone. After 48 h, cells were washed once with phosphate buffered saline (PBS), and incubated for 1 h with or without 200nM human recombinant receptor-associated protein (RAP) (Merck, Darmstadt, Germany) diluted in

complete media. After removing the media, cells were washed once with PBS, and then incubated with 25 μg/ml human recombinant ApoE3 (R&D systems) for 1hr. ApoE was reconstituted with lipid using 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) before the treatment on HepG2 cells[25]. For further analysis, HepG2 cells were washed three times with PBS and harvested with Mammalian Protein Extraction Reagent (Thermo Scientific). The lysate were subjected to SDS-PAGE using anti-human ApoE3 antibody at an 1:150,000 dilution (R&D systems).

12. Statistical analysis

All statistical analyses were conducted using SPSS software (version 18.0; SPSS, Chicago, IL, USA). Values are expressed as the mean ± S.E. Statistical comparisons between groups were performed using the Students' t-test. Data with a p-value < 0.05 were considered significant.

III. RESULTS

1. Rosiglitazone increased LRP1 expression in HepG2 cells in a dose-dependent manner

In HepG2 cells, a human hepatoma-derived cell line, quantitative RT-PCR and immunoblotting showed that LRP1 mRNA and protein expressions were increased by 0.5 and 5 μM of rosiglitazone (Figure 1A and 1B). However, at concentrations above 50 μM of rosiglitazone, LRP1 mRNA and protein expressions did not change compared to those in the non-treated cells (Figure 1A and 1B). We confirmed that rosiglitazone at concentrations up to 5 μM increased the protein amount of LRP1 in HepG2 cells in a dose-dependent manner (Figure 1C).

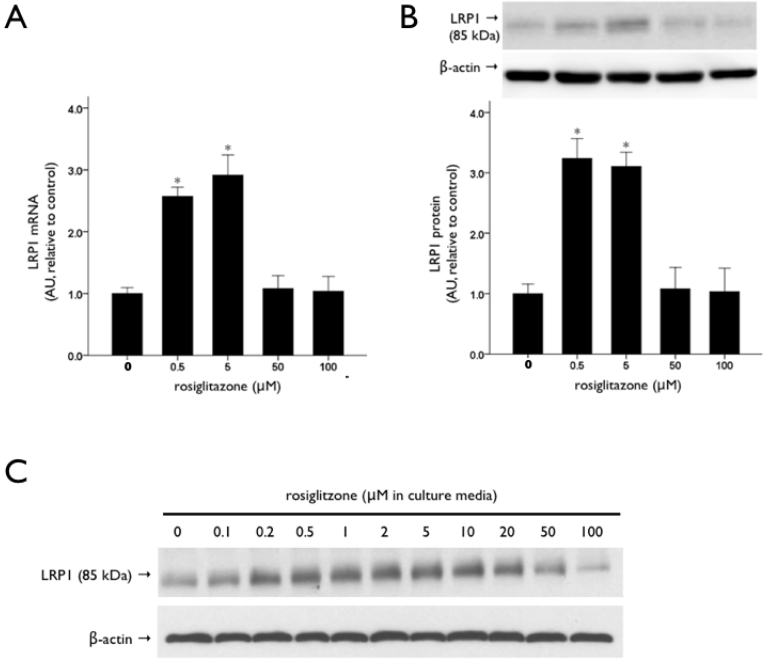

Figure 1. The effect of rosiglitazone on LRP1 expression in HepG2 cells. HepG2 cells were treated with indicated concentrations of rosiglitazone for 48 h. **A,** real-time PCR quantification of LRP1 mRNA in HepG2 cells (n = 6/group). **B,** western blot analysis of LRP1 (β-chain) in HepG2 cells (n = 6/group). **C,** Western blot analysis of LRP1 (β-chain) in HepG2 cells. Results were normalized by β-actin mRNA or protein level and are expressed as ratio relative to non-treated cells. Data are mean ± S.E. *$P < 0.05$ vs rosiglitazone 0 nM.

2. Rosiglitazone induced the binding of PPARγ to a PPRE of the LRP1 promoter in HepG2 cells

To demonstrate the mechanism of LRP1 up-regulation by rosiglitazone, EMSA was performed on the oligomers corresponding to the PPRE sequence of the *LRP1* promoter. The conserved PPRE sequence located at -1185 to -1173 of the LRP promoter[24]. The results presented in Figure 2A showed an increased gel-retarded band in samples derived from the HepG2 cells treated with 0.5 μM of rosiglitazone (lanes 1 and 2). The supershift of this gel-retarded band by the anti-PPARγ antibody supports the involvement of PPARγ in the protein-DNA complex (lane 3). The disappearance of a gel-retarded band in the assays using the excessive unlabeled competitor oligomer and the mutated oligomer shows the involvement of the PPRE sequence of the LRP1 promoter in the protein-DNA complex (lanes 4 and 5).

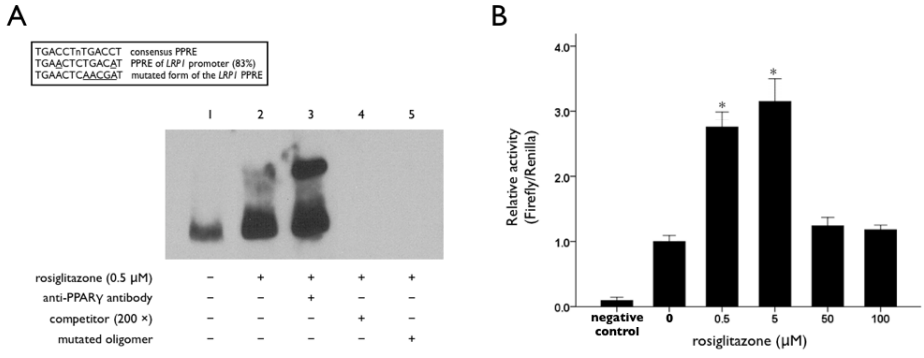

Figure 2. The effect of rosiglitazone on the LRP1 promoter activity in HepG2 cells. HepG2 cells were treated with the indicated concentrations of rosiglitazone for 48 h. **A)** EMSA was performed on oligomers corresponding to a PPRE of the LRP1 promoter. Nuclear extracts were prepared from HepG2 cells without rosiglitazone treatment (lane 1) or with 0.5 μM of rosiglitazone (lanes 2 to 5). Anti-PPARγ antibody was co-incubated before adding the labeled oligomers for a supershift assay (lane 3). Unlabeled oligomers of the LRP1 PPRE at 200-fold molar excess were used to compete with labeled oligomers (lane 4). Labeled oligomers of the mutated form of the LRP1 PPRE were used for specification (lane 5). **B)** The promoter region of human LRP1 was cloned into pGL3-Basic. This construct was transiently co-transfected into HepG2 cells along with the Renilla luciferase reporter vector, pRL-CMV. For the negative control, pGL3-Basic vector was used. Firefly and Renilla luciferase activities were determined using the dual luciferase assay system. The relative luciferase activities were calculated as the ratio of normalized luciferase activities in cells treated with the indicated concentrations of rosiglitazone relative to that of cells without rosiglitazone treatment. Data are mean ± S.E of three independent transfection experiments (each in triplicate reactions). *$p < 0.05$ vs. rosiglitazone 0 nM.

3. Rosiglitazone increased the LRP1 promoter activity in HepG2 cells

The transcription activity of the LRP1 promoter in HepG2 cells was assayed using the promoter-reporter construct. The promoter-reporter construct

contains the LRP1 promoter region including a conserved PPRE. The reporter assay in HepG2 cells showed that rosiglitazone increased the transcriptional activity of the LRP1 promoter at concentrations up to 5 μM (Figure 2B). However, the LRP1 promoter activity in HepG2 cells remained unchanged at rosiglitazone concentrations above 50 μM (Figure 2B).

4. Rosiglitazone increased the uptake apoE in HepG2 cells

We performed an ApoE uptake analysis in HepG2 cells to investigate the function of LRP1 increased by rosiglitazone. After the incubation of HepG2 cells with various concentrations of rosiglitazone for 48 h, the cellular uptake of ApoE during 1 h was measured. We confirmed that endogenous ApoE was not detected in HepG2 cells (Figure 3A). The uptake of ApoE in HepG2 cells was increased by treatment with 0.5 and 5 μM of rosiglitazone (Figure 3A). The increase in the ApoE uptake in HepG2 cells due to rosiglitazone was blocked by the presence of 200 nM RAP, a functional blocker of LRP1 (Figure 3B)

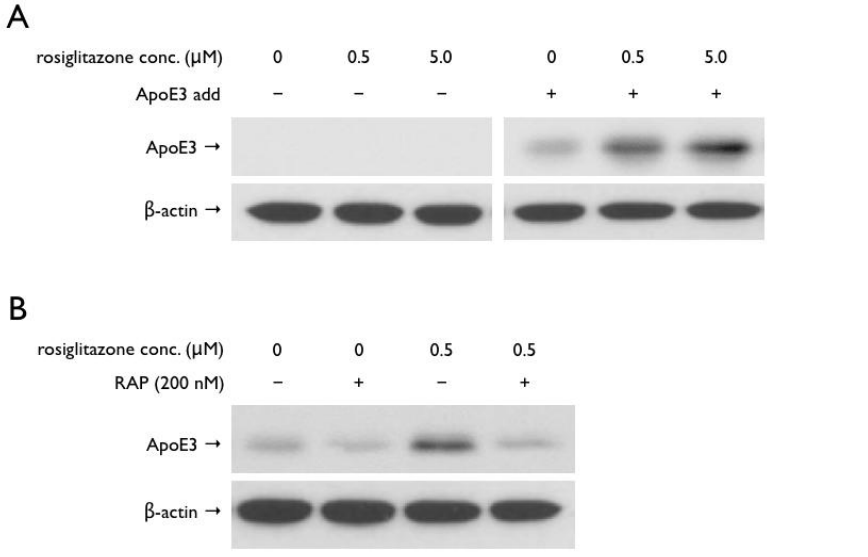

Figure 3. The effect of rosiglitazone on the ApoE uptake in HepG2 cells. HepG2 cells were treated indicated concentrations of rosiglitazone for 48 h. Human recombinant ApoE3 was added to culture media and cells were incubated for 1 h. **A,** Western blot analysis of ApoE3 in HepG2 cells incubated with or without added ApoE3. **B,** Western blot analysis of ApoE3 in HepG2 cells incubated with added ApoE3. Human recombinant RAP was used as a functional blocker of LRP1 and added to culture media prior to adding ApoE3.

5. The LRP1 expression was decreased in the high-fat fed-OLETF rat livers and was recovered by rosiglitazone treatment

To confirm the effect of rosiglitazone on the hepatic LRP1 expression in vivo, we compared the hepatic LRP1 expression among the three experimental animal groups: LETO rats fed a standard chow diet, OLETF rats fed a high-fat diet and high-fat-fed OLETF rats treated with rosiglitazone (4 mg/kg/day for 5 weeks). The results of the OGTT showed the development of DM in high-fat-fed OLETF rats and the improvement in glycemic control in rats treated with rosiglitazone (Figure 4A). The serum triglyceride level was increased in high-fat-fed OLETF rats in comparison to the level in LETO rats and was partially recovered by rosiglitazone treatment (Figure 4B). Interestingly, the protein expression of hepatic LRP1 were reduced in high-fat-fed OLETF rats compared to that of LETO rats, and the rosiglitazone treatment increased the protein expression of hepatic LRP1 in OLETF rats (Figure 5A).

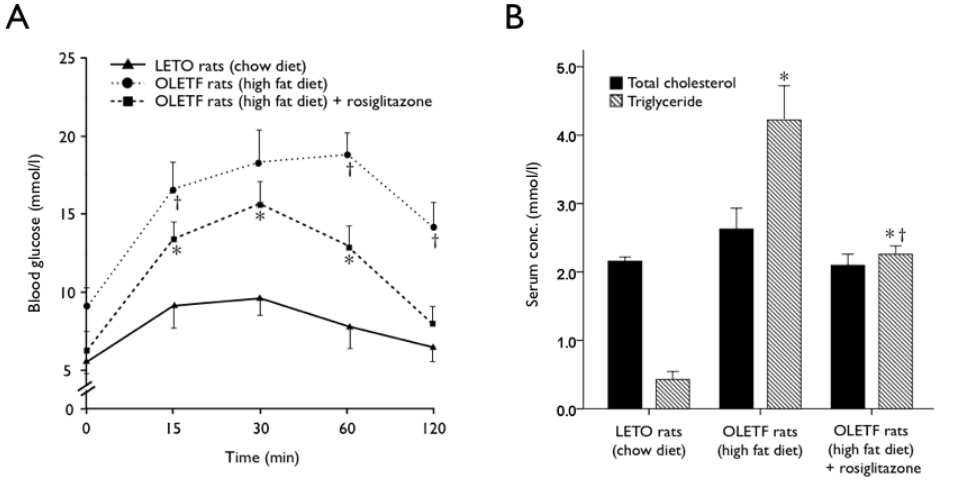

Figure 4. The effect of rosiglitazone treatment on blood glucose and the lipid profile in rats. LETO rats (n = 8) were fed with a normal chow diet and treated with a vehicle; OLETF rats (n = 9) were fed with a high fat diet and treated with a vehicle; OLETF rats (n = 8) were fed with high fat diet and treated with rosiglitazone (4 mg/kg/day) for 5 weeks. **A,** oral glucose tolerance test in each experimental group. **B,** serum concentration of total cholesterol and triglyceride in each experimental group. Data are mean ± S.E. *$P < 0.05$ vs. LETO rats; †$P < 0.05$ vs. OLETF rats.

6. The LRP1 expression in HepG2 cells was decreased under a high glucose condition and recovered by rosiglitazone treatment

To elucidate the factors which decreases the hepatic LRP1 expression, we demonstrated the effect of a high glucose condition on the expression of LRP1 in HepG2 cells. A high glucose condition (25 mM of glucose in culture media) reduced the protein expression of LRP1 in HepG2 cells and the rosiglitazone treatment recovered the LRP1 expression in a high glucose condition (Figure 5B).

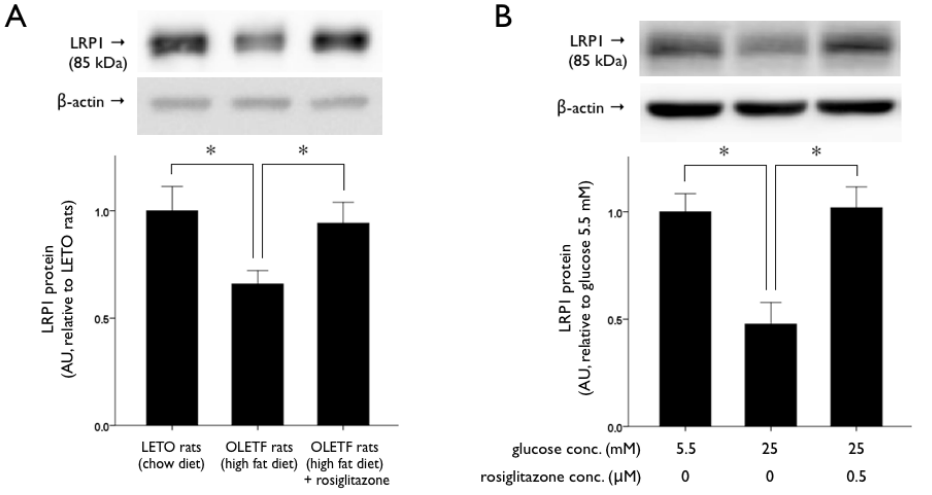

Figure 5. The effect of diabetes and rosiglitazone treatment on hepatic LRP1 expression. A) Western blot analysis of LRP1 (β-chain) in the liver samples of each experimental animal group. LETO rats (n=8) were fed a normal chow diet and treated with a vehicle, while some OLETF rats (n=9) were fed a high fat diet and treated with a vehicle, and the remaining OLETF rats (n=8) were fed a high fat diet and treated with rosiglitazone (4 mg/kg/day) for 5 weeks. **B)** Western blot analysis of LRP1 (β-chain) in HepG2 cells (n=6/group) HepG2 cells were incubated with indicated concentrations of glucose for 24 h and subsequently the cells were treated with indicated concentrations of rosiglitazone for 48 h. Results of western blot analysis were normalized to β-actin protein level and are expressed as ratios relative to LETO rats or to the non-treated cells in a normal glucose (5.5 mM) condition. Data are mean ± S.E. *$p < 0.05$.

IV. DISCUSSION

Recently, Laatsch *et al.*[25] reported that insulin induced the translocation of hepatic LRP1 from the cytosol to the plasma membrane and resulted in an increase in postprandial lipoprotein clearance. They showed that a glucose-induced insulin response increased the hepatic uptake of LRP1 ligands in wild-type mice while insulin-inducible LRP1 ligand uptake was abolished in leptin-deficient obese mice (ob/ob) which are characterised as being hepatic insulin resistance[25]. The commentary on the study of Laatsch *et al.* indicated the clinical mechanistic importance of the study investigating the effects of insulin sensitizing drugs, such as thiazolidinedione, on the metabolism of hepatic LRP1 and remnant lipoproteins[26]. Prior to the study of Laatsch *et al*, an in vitro study reported that rosiglitazone increased LRP1 mRNA transcription, and that the expression of LRP1 is regulated by PPARγ in primary human adipocytes[24]. They reported a conserved PPRE in the promoter region of the *LRP1* gene and showed that the up-regulation of LRP1 by rosiglitazone is mediated by PPARγ and a PPRE in the *LRP1* promoter at a transcriptional level in human adipocytes[24]. If this PPARγ-dependent regulatory mechanism of LRP1 was also effective in hepatocytes, we could suggest a novel mechanism of the beneficial effect of thiazolidinediones on the remnant lipoprotein clearance. Our data showed that rosiglitazone increased the expression of LRP1 in HepG2 cells, and that the 5-week treatment of rosiglitazone increased the hepatic LRP1 expression in OLETF rats. This up-regulation of LRP1 by rosiglitazone increased ApoE uptake in HepG2 cells. We also reaffirmed that the expression of LRP1 is regulated by PPARγ in HepG2 cells. Our *in vitro* and *in vivo* data support our hypothesis suggesting that the hepatic uptake of remnant lipoproteins through LRP1 may contribute to the decrease of serum triglycerides and RLP-c levels due to thiazolidinedione treatment. Considering that this mechanism is not insulin-dependent, increased hepatic LRP1 due to

thiazolidinediones may contribute to the clearance of not only chylomicron remnants in a postprandial status but also VLDL remnants in a fasting status.

In this study, the increases in LRP1 mRNA transcription and protein expression were induced by 100 nM of rosiglitazone and were maintained by up to 5 μM of rosiglitazone in HepG2 cells. At concentrations above 50 μM rosiglitazone, LRP1 expression did not change. This biphasic reaction to rosiglitazone was also seen for *LRP1* promoter activity. This results is consistent with a previous study using adipocytes. In primary human adipocytes, 50 nM rosiglitazone in culture media up-regulated the transcription of LRP1 and the up-regulation was maintained in up to 1 μM rosiglitazone but the induction of the LRP1 expression by rosiglitazone diminished at a concentration of 2 μM[24]. And in SW872 cells, a human liposarcoma cell line, rosiglitazone concentration of 750 nM or higher did not alter the LRP1 mRNA abundance or the LRP1 promoter activity[24]. It was reported that the activation of PPARγ at the AF2 domain by its ligands (PPARγ agonists) increased its transcriptional function, and the same process enhanced subsequent proteosomal degradation of PPARγ[27]. This would explain the reduced efficacy of rosiglitazone at higher concentrations[24]. The pharmacokinetics of rosiglitazone in the human body reveals that the plasma concentration of rosiglitazone reaches the highest level- approximately 300 ng/ml (840 nM)- after a single dose administration of 4 mg[28, 29]. In this regard, the range of rosiglitazone concentration which increased LRP1 in HepG2 cells (100 nM to 5 μM) includes the serum concentration obtained with the conventional use of this drug.

Atherogenic dyslipidemia in DM patients includes high serum triglyceride and low serum HDL-c levels and this alteration in the lipid profile has been explained by an increase in hepatic VLDL synthesis resulting from insulin resistance[1-3]. Our animal data showed that hepatic LRP1 was reduced in diabetic OLETF rats compared to levels in non-diabetic LETO rats. This result suggests that the alteration in hepatic clearance of triglyceride-rich remnant

lipoproteins through LRP1 may be associated with the development of atherogenic dyslipidemia in a diabetic condition. The alteration of LRP1 in a diabetic status has been reported in one study investigating the blood-brain barrier[30], in which, the expression of LRP1 in the brain microvessel was reduced in streptozotocin-induced diabetic mice[30]. Because a streptozotocin-induced diabetic animal model is characterized by high serum glucose rather than insulin resistance, we investigated the effect of a high glucose condition on the LRP1 expression in HepG2 cells. As expected, a high glucose condition decreased the expression of LRP1 in HepG2 cells. Our data suggest that hyperglycemia can be associated with decrease in hepatic LRP1 and alter the clearance of remnant lipoproteins from circulation. Considering the alteration in the hepatic LRP1 translocation to the plasma membrane in an insulin-resistant animal model[25], the hepatic clearance of remnant lipoproteins through LRP1 in a diabetic condition might be reduced by a decrease in LRP1 itself as well as a decrease in the functional efficiency of LRP1. However, our data cannot exclude the possibility that factors associated with insulin resistance altered the hepatic LRP1 expression. The mechanism of the decrease in hepatic LRP1 in a diabetic status should be elucidated in future studies.

One of the limitations of this study is the lack of the serum RLP-c data. Currently available commercial kits are able to measure only human serum RLP-c levels. However, a strong correlation between serum RLP-c and serum triglyceride level has been reported[31]. Another limitation of this study is that we did not demonstrate the direct association of altered hepatic LRP1 with serum triglyceride level *in vivo*. We observed an increase in serum triglyceride level along with decreased hepatic LRP1 in diabetic rats and a decrease in serum triglyceride level with increased hepatic LRP1 after rosiglitazone treatment. However, because other mechanisms which are associated with the change in serum triglyceride level have been illustrated in diabetes[3] and thiazolidinedione treatment[23], our *in vivo* data cannot conclude a causal relationship between the

hepatic LRP1 expression and serum triglyceride level. Nevertheless, we demonstrated that ApoE uptake through LRP1 is induced by rosiglitazone in a human hepatoma-derived cell line, and these data suggest that a change in hepatic LRP1 can affect the clearance of triglyceride-rich remnant lipoproteins and serum triglycerides level.

V. CONCLUSION

In this study, rosiglitazone treatment increased LRP1 expression and function in HepG2 cells and in the livers of diabetic rats. In addition, a diabetic condition including a high glucose condition decreased the expression of hepatic LRP1. These data suggest that decreased hepatic LRP1 in a diabetic condition is associated with the development of atherogenic dyslipidemia and that increased hepatic LRP1 by thiazolidinediones contributes to an improvement in atherogenic lipid profiles in diabetic patients.

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ABSTRACT(IN KOREAN)

로지글리타존이 간의 LRP1 발현에 미치는 영향: 티아졸리딘디온 제제가 당뇨병에서 발생하는 죽상경화성 지질대사이상을 호전시키는 새로운 기전

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문 재 훈

간에서 발현되는 low-density lipoprotein receptor-related protein 1 (LRP1)은 혈중의 remnant lipoprotein 을 청소하여 그 혈중농도를 감소시키는 데 있어 중요한 역할을 하는 수용체이다. 티아졸리딘디온 약제는 당뇨병 환자에서 인슐린저항성을 개선시켜 혈당을 감소시키면서 동시에 지질 대사의 개선에도 효과가 있는 것으로 알려져 있다. 본 연구에서는 티아졸리딘디온 약제가 지질 대사에 작용하는 기전을 연구하기 위해 티아졸리딘디온 제제가 간의 LRP1 발현과 기능에 미치는 영향에 대해 조사하였다.

세포실험을 위해 HepG2 세포를 여러 농도의 로지글리타존을 처리하여 배양하였으며 Otsuka Long-Evans Tokushima Fatty (OLETF) rat 을 당뇨 동물 모델로, Long-Evans Tokushima Otsuka (LETO) rat 을 정상 동물 모델로 사용하였다. 동물에 5 주간 로지글리타존을 투여하여 HepG2 세포 및 동물의 간조직에서 LRP1 의 발현과 기능을 분석하였다.

0.5 와 5μM 의 로지글리타존 처리에 의해 HepG2 세포에서

LRP1 의 mRNA 와 단백질 발현이 증가하였으나 50μM 이상의 농도에서는 로지글리타존 처리를 하지 않은 세포와 비교하여 LRP1 의 mRNA 와 단백질의 발현에 차이를 보이지 않았다. 로지글리타존이 peroxisome proliferator-activated receptor-γ 를 활성화시켜 LRP1 promoter 에 존재하는 perxisome proliferator response element 에 결합하게 하여 LRP1 promoter 의 전사활성을 증가시킴을 HepG2 세포에서 electromobility shift assay 와 reporter assay 를 통해 보였다. 로지글리타존에 의한 전사활성 증가 효과는 LRP1 의 mRNA 나 단백질의 발현 증가 효과와 같은 양상으로 나타났다. 또한 HepG2 세포에서 로지글리타존에 의해 LRP1 을 통한 ApoE 의 세포내 이동이 증가함도 확인하였다. OLETF rat 에서는 LETO rat 에 비해 혈중 중성지방 농도가 높게 나타났고 로지글리타존 투여에 의해 부분적으로 회복되었다. 간의 LRP1 발현은 OLETF rat 에서 LETO rat 에 비해 감소되었으며 로지글리타존 투여에 의해 OLETF rat 의 간에서 LRP1 발현이 증가하였다. 이에 더해, 정상조건에 비해 고혈당 조건에서 배양한 HepG2 세포에서 LRP1 의 발현이 감소하고 로지글리타존에 의해 LRP1 발현이 정상조건의 HepG2 세포 수준까지 증가함도 확인하였다.

결론적으로 본 연구 결과는 당뇨 조건에서 간의 LRP1 발현 감소가 죽상경화성 지질대사이상의 발생과 관련 있고 티아졸리딘디온 제제에 의한 간의 LRP1 발현증가가 당뇨병 환자에서 이러한 죽상경화성 지질대사이상의 호전에 기여함을 시사한다.

핵심되는 말

LRP1, 당뇨병, 티아졸리딘디온 제제, 죽상경화성 지질대사이상, PPARγ