# The effect of PPAR- γ agonist (Rosiglitazone) and Angiotensin II receptor blocker (Losartan) on the myocardial structure and function assessed with two-dimensional echocardiography and strain rate imaging in type 2 diabetic OLETF

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Directed by Professor Namsik Chung

The Doctoral Dissertation

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degree of Doctor of Philosophy

Chi Young Shim

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This certifies that the Doctoral Dissertation

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# <TABLE OF CONTENTS>

ABSTRACT·····1

I. INTRODUCTION·····4

II. MATERIALS AND METHODS·····6

1. Experimental animals, drug regimens and sample preparation····6

2. Plasma glucose, insulin and lipids measurement·····8

3. Reverse Transcription–Polymerase Chain Reaction·····8

A. Isolation of total RNA·····8

B. cDNA synthesis ·····9

C. PCR analysis ·····9

4. Massion's trichrome staining·····10

5.Immunostaining·····10

6. Echocardiographic analysis·····11

A. Two-dimensional echocardiography ·····11

B. Analysis of strain and strain rate imaging·····12

7. Statistical analysis·····12

III. RESULTS ·····13

1. Body weights and heart weight ·····12

2. Serologic and urinary findings·····14

3. Expression of cytokine gene in cardiac tissue ·····15

4. Histology and determination of fibrosis area····· 17

5. Comparison of collagen type I and III in left ventricle ····· 18

6. Evaluation of cardiac function ····· 18

IV. DISCUSSION····· 21

V. CONCLUSION·····23

REFERENCES …… 24

ABSTRACT (IN KOREAN) ……29

<LIST OF FIGURES, LIST OF TABLES>

# LIST OF FIGURES

Figure 1. Expression levels of cytokine genes determined by RT-PCR among the groups ·····16

Figure 2. Representative myocardial histologic findings and the degree of interstitial fibrosis in experimental animals ·····17

Figure 3. Interposition of collagen fibers in experimental animals (*upper*, collagen type I; *lower*, collagen type III) ····· 18

# LIST OF TABLES

Table 1. Primer sequences for RT-PCR analysis ·····10

Table 2. Data of body weight and heart weight at baseline (28 weeks) and after treatment (at 40 weeks) ·····13

Table 3. Data of plasma glucose, plasma insulin, plasma lipids and urine albumin/creatinine ratio at baseline (28 weeks) and after treatment (at 40 weeks) ····· 14

Table 4. The myocardial structure and function assessed with

two-dimensional echocardiography and strain rate imaging at baseline (28 weeks) and after treatment (at 40 weeks) ·····19

<ABSTRACT>

# The effect of PPAR- γ agonist (Rosiglitazone) and Angiotensin II receptor blocker (Losartan) on the myocardial structure and function assessed with two-dimensional echocardiography and strain rate imaging in type 2 diabetic OLETF

**Chi Young Shim**

***Department of Medicine***

***The Graduate School, Yonsei University***

**(Directed by Professor Namsik Chung)**

Myocardial dysfunction is more frequently observed in diabetic patients with albuminuria. The aim of this study was to investigate the effect of the peroxisome proliferator-activated receptor (PPAR) ligands and the angiotensin II receptor blocker (ARB) on myocardial structure and function and the association with the degree of albuminuria in the type 2 diabetic rat model. Five male Long-Evans Tokushima Otsuka (LETO) rats and 20 male Otsuka Long-Evans Tokushima Fatty (OLETF) rats were used. The animals were divided into five groups at 28 weeks of age as follows: untreated LETO rats, untreated OLETF rats, OLETF rats treated with losartan, OLETF rats treated with rosiglitazone and OLETF rats treated with a combination of both

drugs. The ARB, losartan was given in the dose of 5 mg/kg/d and the PPAR-gamma agonist, rosiglitazone was given in the dose of 3 mg/kg/d, by oral gavage for a subsequent 12 weeks. At 28 weeks and at 40 weeks, urine samples and blood samples were collected and two-dimensional echocardiogram and strain rate imaging were obtained for the assessment of myocardial structure and function. At the end of the experimental period, hearts were rapidly excised for histological analysis.

The OLETF rats showed increased fasting glucose levels, fasting insulin levels and urine albumin/creatinine ratio compared with the LETO rats not only at 28 weeks but also at 40 weeks. At 40 weeks, it showed better metabolic profiles in the rosiglitazone group and in the combination group than in the untreated or the losartan group. The losartan-treated or combination drug-treated OLETF rats showed a significant attenuation in the progression of albuminuria. The expression of growth factors (TGF-β and TNF-α) and proinflammatory cytokines (IL-1 and IL-6) in cardiac tissue increased significantly in the OLETF rats compared with the LETO rats. After treatment with losartan or combination with losartan plus rosiglitazone, the expression those growth factors and cytokines in the OLETF cardiac tissue attenuated. In the cardiac structure and functional analysis, the histological results including a degree of interstitial fibrosis and collagen interposition of left ventricle revealed that interstitial fibrosis was also attenuated in the losartan group and the combination group than the untreated OLETF group. I could see consistent results in the comparison of collagen type I and III expression in left ventricle and the echocardiographic parameters assessed

with two-dimensional echocardiography and strain rate imaging. In conclusion, the ARB, losartan revealed protective effect on the progression of albuminuria, myocardial structural and functional changes. The PPAR-r agonist, rosiglitazone showed minimal protective effect on the progression of albuminuria and no significant myocardial structural and functional benefits even though it had a metabolic benefits. The combination of losartan and rosiglitazone showed both metabolic benefits and myocardial protective effects.

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Key words: angiotensin II receptor blocker, peroxisome proliferator-activated receptor- γ agonist, diabetes mellitus, albuminuria, myocardium

# The effect of PPAR- γ agonist (Rosiglitazone) and Angiotensin II receptor blocker (Losartan) on the myocardial structure and function assessed with two-dimensional echocardiography and strain rate imaging in type 2 diabetic OLETF

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## I. INTRODUCTION

The principal pathophysiological feature of diabetes mellitus is the morphological and functional alteration of microvessels, called diabetic microangiopathy. Patients with diabetes mellitus are characterized by an increased likelihood of heart failure. The mechanisms of myocardial dysfunction in diabetic patients are multifactorial and include metabolic disturbances, myocardial fibrosis, small vessel disease (so called microangiopathy), autonomic dysfunction, and insulin resistance. Also, previous investigators demonstrated that the expression of growth factors such as TGF-β and TNF-α or the proinflammatory cytokines such as IL-1 and IL-6 had an important role in the development of diabetic complications.

The patients with microalbuminuria or proteinuria have 2-10 times faster progression of cardiac and vascular complications, compared with subjects with normal albumin secretion. Albuminuria reflects a renal and systemic transvascular albumin leakage, therefore it has been considered a marker of microangiopathy. Diabetic myocardial dysfunction is more frequently observed in patients with microalbuminuria. But, the association of microalbuminuria on myocardial dysfunction is not understood clearly and the causes of development of combined renal and myocardial complications in type 2 diabetes are poorly understood. The microangiopathic complications of diabetes such as diabetic cardiomyopathy and diabetic nephropathy usually have a prolonged, asymptomatic phase characterised by early subclinical functional and structural abnormalities until these are presented as congestive heart disease and renal failure. Early detection and medical intervention of these subclinical pathologic status are very important clinically.

Angiotensin II, the effector molecule of the renin-angiotensin system, has profound effects on endothelial and smooth muscle cells. These effects are not only hemodynamic in nature, but also comprise inflammation, thrombosis, and cell proliferation through stimulation of production of cytokines and growth factors. In diabetes mellitus, these effects seem amplified with adverse consequences like atherosclerosis and occlusive microangiopathy. Suggestive evidence for this notion is the impressive beneficial effect of pharmacological interference with the renin-angiotensin system in large vessel disease as well as in renal and retinal microangiopathy. The action of angiotensin converting enzyme (ACE) inhibitors on angiotensin II may improve fibrosis in

myocardium and functional and structural changes of small vessels in diabetes. Angiotensin II receptor blockers (ARBs) may also have similar effects on microangiopathy and myocardial fibrosis in diabetic patients.

PPAR- γ agonists have a well-established therapeutic role for treating type 2 diabetes mellitus. Favorable renal effects have also been seen, including both indirect systemic effect and direct renal effects. PPAR-γ treatment has been associated with reduction in microalbuminuria in patients with type 2 diabetes. In keeping with the observations that PPAR-γ is present in glomerular mesangial cells, these data are consistent with direct renal action in diabetic kidney. So, PPAR-γ agonists can improve urine albumin excretion and slow the progression of glomerulosclerosis in animal models.

These desirable renal and myocardial effects make ARBs and PPAR-γ agonists promising targets for treating not only diabetic nephropathy but also diabetic cardiomyopathy in aspect of treatment for microangiopathy. Therefore, we sought to investigate the effect of rosiglitazone (a PPAR-r agonist) and losartan (an ARB) on myocardial function as well as structural change of myocardium and microvasculature and the association with the degree of microalbuminuria in the type 2 diabetic rat model.

## II. MATERIALS AND METHODS

### 1. Experimental animals, drug regimens and sample preparation

All procedures were in accordance with institutional guidelines for animal research. Twenty male Otsuka Long-Evans Tokushima Fatty (OLETF) rats aged 4 weeks, which are established as spontaneously long-term

hyperglycemic rats with type II diabetes mellitus and five male Long-Evans Tokushima Otsuka (LETO) rats aged 4 weeks were obtained from the Tokushima Research Institute of Otsuka Pharmaceutical Co (Japan) and maintained in an animal facility with ventilation, controlled temperature (22°C ± 2°C), and a 12-hour light/dark cycle (lights on at 8:00 AM). All animals were housed in the same plastic cages (*n* = 2 per cage) and were fed ad libitum with standard chow diet and tap water.

A total of 5 LETO rats and 20 OLETF rats were used. The OLETF rats were randomly divided into four groups (n = 5 per group) at 28 weeks of age: Group I = untreated LETO rats ; Group II = untreated OLETF rats ; Group II = OLETF rats treated with losartan ; Group III = OLETF rats treated with rosiglitazone; Group IV = OLETF rats treated with a combination of both drugs. The ARB, losartan (Merck & Co., Readington Township, NJ, U.S.A) was given in the dose of 5 mg/kg body weight per day by oral gavage and the PPAR-γ agonist, rosiglitazone (GlaxoSmithKline plc., London, U.K) was given in the dose of 3 mg/kg body weight per day, also by oral gavage for a subsequent 12 weeks. Urine samples and blood samples were collected at 28 weeks and at 40 weeks of age. Urine samples were collected in metabolic cages at least 10 μl/ml. At the end of the experimental period (at 40 weeks of age), the animals were anesthetized and the trunk blood was collected to measure serologic parameters. In addition, hearts were rapidly excised and weighted after removing the blood by squeezing.

2. Plasma glucose, insulin and lipids measurement

The plasma glucose levels were measured using the glucose oxidase method. The plasma insulin concentrations were measured by a radioimmunoassay using a double-antibody method with a commercially available radioimmunoassay kit. The plasma total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein levels were measured by an enzymatic colorimetric method using commercially available kits. For measuring fasting plasma glucose levels and plasma insulin concentrations, blood samples were obtained after overnight fasting.

3. Reverse Transcription–Polymerase Chain Reaction (Cytokine gene expression assay)

A. Isolation of total RNA

Total RNA was extracted by 500 μl/60 mm plate Tri-reagent (Sigma, Missouri, JC, U.S.A). Chloroform was poured about 100 μl above Tri-reagent, voltexing a sample about 10 seconds. Then, sample was centrifuged at 12000 g, 4°C and 15 mins. Three layers were appeared in the tube, transparency upper layer collected in new tubes. And, 2-propanol was poured about 250 μl over the sample, again voltex a sample about 30 secs. Centrifuge was accomplished about 12000 g, 4°C and 10 mins. Left the pellet, supernatant was discarded and washed by 75% ethanol (Duksan, Seoul, Korea) –mixed diethylpyrocarbonate (DEPC; Sigma) water. Centrifugation was also operated about 7500 g, 4°C and 5 mins. The supernatant was dismissed, and pellet was dried on room temperature about 7 mins. Finally, 30 μl nuclease free water

(NFW) was poured onto pellet. The quality and quantity of the RNA was detected by $OD_{260}/OD_{280}$ with DU 640 spectrophotometer (Effendorf, Hamburg, Germany).

B. cDNA synthesis

Complementary DNA (cDNA) was synthesized with RT-&GO$^{TM}$. Quantitative 1 μg total RNA was added to 1 μl anchored primer $(dT)_{25}V$, 2 μl dithiothreitol (DTT) and NFW, totally 9 μl. To prevent secondary structures, mixture was incubated for 5 mins at 70°C, and added 8 μl of RT-&GO$^{TM}$ mastermix. Sample was incubated the assay at 42°C for 1 hr. At the conclusion, sample was inactivated the reverse transcriptase at 70°C for 15 mins. Alike isolation of total RNA, sample was detected by $OD_{260}/OD_{280}$ with DU 640 spectrophotometer.

C. PCR analysis

Quantitative 1 μg cDNA, each 10 pM primer (forward and reverse; Table 1), 0.1 mM dNTP mixture, 1.25 U of Taq polymerase and 10 × reaction buffer were mixed with NFW, lastly total volume of 25 μl. PCR condition was fixed as fellow. A cycle of denatureing at 94°C for 3 minutes followed by number of 35 cycles with denaturation at 94°C for 30 secs, annealing at 48°C to 60°C for 30 secs, and elongation at 72°C for 30 secs. Then sample was kept up 72°C for 10 mins. When PCR assay have finished, PCR product was separated by electrophoresis in a 1.2% agarose gel (Biorad, Hercules, CA, U.S.A) and Gel-Doc (Biorad) visualized after staining with ethidium bromide

(EtBr; Sigma).

Table 1. Primer sequences for RT-PCR analysis

| Primer | Sequence (5' → 3') | Primer | Sequence (5' → 3') |
|---|---|---|---|
| IL-1β | TGACCCATGTGAGCTGAAAG<br>AGGGATTTTGTCGTTGCTTG | IL-6 | GACTGATGTTGTTGACAGCCACTGC<br>TAGCCACTCCTTCTGTGACTCTAACT |
| TGF-β | CTTCAGCTCCACAGAGAAGAACTGC<br>CACGATCATGTTGGACAACTGCTCC | TNF-α | GTAGCCCACGTCGTAGCAAA<br>CCCTTCTCCAGCTGGGAGAC |
| 18s rRNA | GTCCCCCAACTTCTTAGAG<br>CACCTACGGAAACCTTGTTAC | | |

4. Massion's trichrome staining

The heart was perfusion-fixed with 10% (vol/vol) neutral buffered formaldehyde for 24 hrs, transversely sectioned into four comparably thick sections, and embedded in paraffin by routine methods. Sections of 5-μm thickness were mounted on gelatin-coated glass slides to ensure different stains could be used on successive sections of tissue cut. After deparaffinization and rehydration, the sections were stained with massion's trichrome staining to assess cytologic details such as interstitial fibrosis. Interstitial fibrosis area was measured with MetaMorph software version 4.6 (Universal Imaging Corp., Downingtown, PA, U.S.A).

5. Immunostaining

Histological analysis was performed according to the instructions of the manufacturer (R.T.U VECTASTAIN Universal Quick Kit, Vector

Laboratories). In brief, the excised heart tissues were fixed in 3.7% buffered formaldehyde and embedded in paraffin. Tissue sections, 5 μm thick, were deparaffinized, rehydrated and rinsed with PBS. Sodium citrate antigen retrieval was experimented with 10 mM sodium citrate (pH 6.0) in microwave for 10 mins. Sections were incubated in 3% $H_2O_2$ in order to quench endogenous peroxidase. Sample was blocked in 2.5% normal horse serum, and incubated in collagen type Ⅰand Ⅲ. Biotinylated pan-specific universal secondary antibody and streptavidin/peroxidase complex reagent was treated with heart section. Using DAB substrate kit, heart section was stained with antibody. Counterstain was operated in 1% methyl green and dehydration was progressed with 100% N-butanol (Duksan), ethanol and xylene (Duksan).

6. Echocardiographic analysis (Assessment of cardiac function)

A. Two-dimensional echocardiography

Transthoracic echocardiographic studies were performed on a GE Vivid 7 ultrasound machine (GE Medical System, Schenectady, NY, U.S.A) with a 10.0 MHz transducer at 28 weeks of age (before randomization of groups) and at 40 weeks of age (at the end of the experimental period) by an experienced cardiologist who was blinded to the group to which the animals had been allocated. The rats were anesthesed generally with inhaled isoflurane. The chest was shaved and the rats were placed in the left lateral decubitus position. The transducer was placed on the left hemithorax and short axis views were recorded. Two-dimensional images were obtained at mid papillary level. The M-mode tracing of left ventricular (LV) contraction was also obtained at the

same level as the short-axis view. The LV end diastolic dimension (LVEDD) and LV end systolic dimension (LVESD) were measured with the M-mode tracing. These parameters allowed the left ventricular fractional shortening (FS) to be calculated using the equation FS = [(LVEDD-LVESD)/ LVEDD] × 100%. Two images were obtained in each view and each parameter was measured from 3 consecutive beats in each image. Six values of each parameter were measured and the average was recorded.

B. Analysis of strain and strain rate imaging

Echocardiograms were stored digitally and analyzed offline with EchoPAC 6.3.0 software (GE Vigmed, Horten, Norway) with custom two-dimensional strain rate imaging software. More than three images were obtained in short axis view, and the parameters were measured from 3 consecutive beats in each image. For quantitative analysis of global LV systolic function, peak radial systolic strain and systolic strain rate at six segments (anteroseptum, anterior, anterolateral, posterolateral, inferior, inferoseptum) were obtained and the average values were calculated.

7. Statistical analysis

Data are expressed as means ± standard deviation. Statistical comparisons between the two groups were performed using the Student's *t*-test. In addition, a one-way ANOVA was used when comparing more than two groups. A $p$-value < 0.05 was considered significant.

## III. RESULTS

1. Body weights and heart weight

The OLETF rats had a higher body weight than the LETO rats at 28 weeks of age. At 40 weeks of age, the body weight of the OLETF rats was significantly higher than the LETO rats. In addition, the body weight of OLETF rats treated with rosiglitazone was higher than those treated with losartan and combination drugs. There were no significant differences in the heart weight and left ventricle/ heart weight ratio between groups.

Table 2. Data of body weight and heart weight at baseline (28 weeks) and after treatment (40 weeks)

| | LETO (n = 5) | OLETF (n = 20) | | | |
|---|---|---|---|---|---|
| | | CON (n = 5) | LOS (n = 5) | ROS (n = 5) | LOS + ROS (n = 5) |
| Body Wt 28 weeks (g) | 504 ±14 | 548 ± 14 * | 552 ± 37 * | 549 ± 25 * | 561 ± 38 * |
| Body Wt 40 weeks (g) | 511 ± 31 | 616 ± 40 * | 571 ± 27 *† | 645 ± 42 *† | 612 ± 31* |
| Change of body Wt (g) | 7 ± 7 | 67 ± 25 * | 20 ± 13 *† | 97 ± 22 *† | 51 ± 30* |
| Heart Wt 40 weeks (g) | 2.0 ± 0.1 | 2.0 ± 0.1 | 2.4 ± 0.1 | 2.1 ± 0.1 | 2.3 ± 0.2 |
| LV/heart Wt 40 weeks (%) | 0.7 ± 0.0 | 0.7 ± 0.1 | 0.7 ± 0.0 | 0.7 ± 0.1 | 0.7 ± 0.1 |

CON, untreated OLETF controls; LOS, OLETF rats treated with losartan; ROS, OLETF rats treated with rosiglitazone; LOS + ROS, OLETF rats treated with losartan and rosiglitazone; Wt, weight;g, gram

* $p$ <0.05, compared with the untreated LETO rats.

† $p$ <0.05, compared with the untreated OLETF rats.

2. Serologic and urinary findings

The OLETF rats showed increased fasting glucose level, fasting insulin level and urine albumin/creatinine ratio compared with the LETO rats not only at 28 weeks but also at 40 weeks. There were no significant differences in these serologic and urinary parameters among the groups of OLETF rats at 28 weeks. At 40 weeks, it showed better metabolic profiles in the rosiglitazone group and in the combination group than in the untreated or the losartan group. The losartan-treated or combination drug-treated OLETF rats showed a significant attenuation in the progression of albuminuria.

Table 3. Data of plasma glucose, plasma insulin, plasma lipids and urine albumin/creatinine ratio at 28 weeks and after treatment (at 40 weeks)

| | LETO (n = 5) | OLETF (n = 20) | | | |
|---|---|---|---|---|---|
| | | Control (n = 5) | Los (n = 5) | Ros (n = 5) | Los + Ros (n = 5) |
| **At 28 weeks** | | | | | |
| Fasting glucose (mg/dl) | 92 ± 12 | 124 ± 20 * | 126 ± 17 * | 129 ± 25 * | 128 ± 21 * |
| Fasting insulin (ng/ml) | 1.9 ± 0.8 | 6.1± 1.9 * | 5.9± 1.0 * | 6.9± 2.6 * | 5.9± 1.3 * |
| Total Chol (mg/dl) | 93 ± 5 | 102 ± 11 | 99 ± 13 | 104 ± 15 | 102 ± 10 |
| Triglyceride (mg/dl) | 21 ± 10 | 33 ± 15 | 31 ± 14 | 33 ± 17 | 32 ± 9 |
| HDL Chol (mg/dl) | 25 ± 4 | 25 ± 9 | 24 ± 7 | 24 ± 8 | 25 ± 6 |
| LDL Chol | 14 ± 1 | 17 ± 2 | 16 ± 3 | 17 ± 3 | 15 ± 1 |

| | | | | | |
|---|---|---|---|---|---|
| (mg/dl) | | | | | |
| Urine Alb (mg/dL) | 0.46 ± 0.1 | 8.2 ± 0.8 * | 4.8 ± 0.2 * | 4.5± 1.3 * | 3.6 ± 0.1* |
| Urine Cr (mg/dL) | 95± 81 | 168 ± 47 | 178± 172 | 120 ± 120 | 109 ± 98 |
| Urine Alb/Cr | 5.4 ± 2.8 | 77.4 ± 58.1* | 32.0 ± 12.9* † | 45.9 ± 72.5* † | 27.7 ± 22.3 *† |
| **At 40 weeks** | | | | | |
| Fasting glucose (mg/dl) | 103 ± 12 | 161 ± 31 * | 153 ± 23 * | 130 ± 30 *† | 129± 18 *† |
| Fasting insulin | 2.4 ± 1.0 | 9.8 ± 3.0 * | 7.0± 1.6 * | 4.6± 2.6 † | 3.8± 1.0 † |
| Total Chol (mg/dl) | 93 ± 5 | 98 ± 18 | 103 ± 4 | 104 ± 13 | 101 ± 13 |
| Triglyceride (mg/dl) | 21 ± 10 | 112 ± 25 | 55 ± 17 | 39 ± 17 | 35 ± 4 |
| HDL Chol (mg/dl) | 25 ± 4 | 24 ± 2 | 23 ± 5 | 27 ± 9 | 22 ± 2 |
| LDL Chol (mg/dl) | 14 ± 1 | 23 ± 6 | 18 ± 1 | 18± 2 | 16 ± 3 |
| Urine Alb (mg/dl) | 0.46 ± 0.1 | 8.2 ± 0.8* | 4.8 ± 0.2* | 4.5± 1.3* | 3.6 ± 0.1* |
| Urine Cr (mg/dl) | 95± 81 | 168 ± 47 | 178± 172 | 120 ± 120 | 109 ± 98 |
| Urine Alb/Cr | 5.4 ± 2.8 | 77.4 ± 58.1 * | 32.0 ± 12.9 *† | 45.9 ± 72.5 *† | 27.7 ± 22.3 *† |

LOS, Losartan; ROS, Rosiglitazone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Chol, cholesterol; Alb, albumin; Cr, creatinine

* $p$ <0.05, compared with the untreated LETO rats.

† $p$ <0.05, compared with the untreated OLETF rats.

3. Expression of cytokine gene in cardiac tissue

Using reverse transcription-polymerase chain reaction **(**RT-PCR**)** analysis, we could compare the expression of cytokine gene of myocardium. The growth factors such as TGF- β (transforming growth factor), TNF- α (Tumor

necrosis factor) and proinflammatory cytokines such as IL (interleukine)-1β and IL-6 were significantly increased in the OLETF rats compared with the LETO rats. However, the expression of each gene was significantly (2 or 4 folds) attenuated in the losartan-treated and the combination groups compared with the untreated OLETF rats. So, after treatment with losartan or combination with losartan plus rosiglitazone, the expression of cytokine gene in the OLETF cardiac tissue became similar to that of the LETO cardiac tissue.

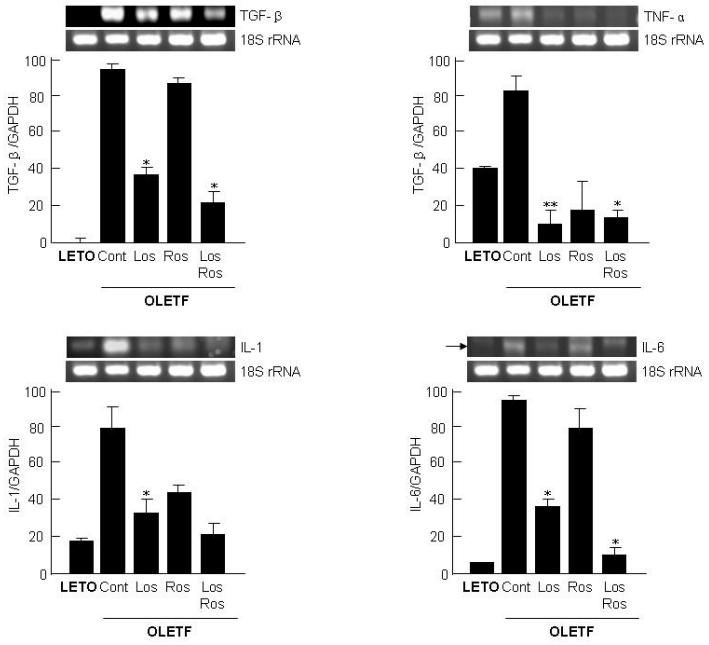


Figure 1. Expression levels of cytokine genes determined by RT-PCR among the groups.

4. Histology and determination of fibrosis area

The dimensions of left ventricle were larger in the untreated OLETF and rosiglitazone-treated OLETF rats than those in LETO rats. However, in the OLETF rats treated with losartan and combination drugs, the cardiac dimensions were similar with those of LETO rats. Interstitial fibrosis was attenuated about 3% in the OLETF rats treated with losartan and combination drugs than the untreated OLETF rats.

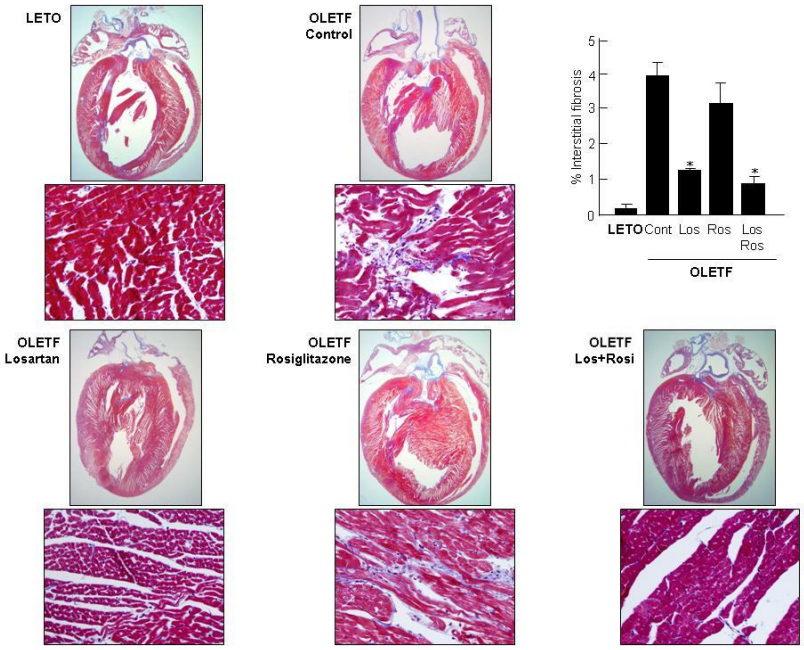


Figure 2. Representative myocardial histologic findings and the degree of interstitial fibrosis in experimental animals.

5. Comparison of collagen type I and III in left ventricle

The collagen interposition of left ventricle was increased in the untreated OLETF controls. In groups treated with losartan or combination of both drugs , I could see similar histologic findings in the LETO rats.

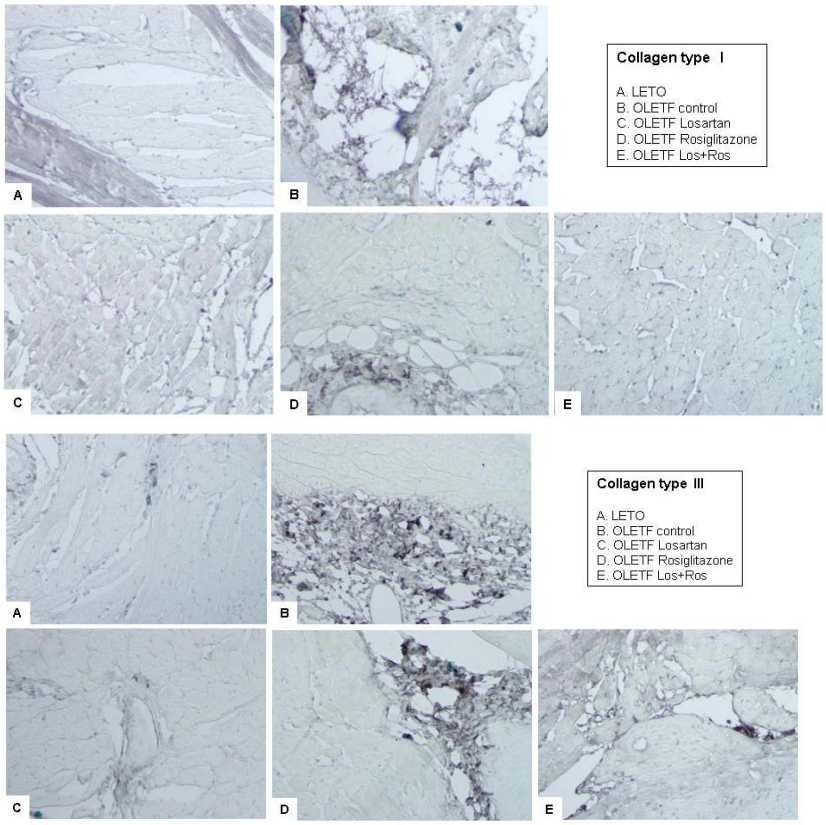


Figure 3. Interposition of collagen fibers in experimental animals (*upper*, collagen type I; *lower*, collagen type III).

6. Evaluation of cardiac function

Cardiac dimensions and performance parameters were measured by transthoracic echocardiography. At 28 weeks, echocardiographic parameters

including left ventricular end-diastolic dimension, end-systolic dimension was slightly larger in OLETF rats than LETO rats but there were no statistical difference. Although there were no difference in the left ventricular ejection fraction, the more senditive parameters, the global radial strain and strain rate, were significantly smaller in OLETF rats. It suggested that there was subclinical left ventricular systolic dysfunction in diabetic rats at the age of 28 weeks.

After 12 weeks (from 28 weeks to 40 weeks) treatment, we repeatedly performed transthoracic echocardiogram for assessment of the effects of losartan, rosiglitazone and losartan + rosiglitazone on cardiac function. In the untreated OLETF rats, the left ventricle was progressively dilated and myocardial function was more decreased. In the OLETF rats treated with losartan or combination with both drugs, the global radial strain and strain rate assessed with strain rate imaging, left ventricular ejection fraction and chamber size became similar to those in the untreated LETO rats. However, the OLETF rats treated with rosiglitazone revealed increased cardiac size and reduced systolic function. From these findings, we can suggest that rosiglitazone only treatment showed no beneficial effect on the protection of progressive decrement in global myocardial function in diabetic rats. But interestingly, the combination of both drugs can revealed favorable effect on myocardial function.

Table 4. The myocardial structure and function assessed with two-dimensional echocardiography and strain rate imaging at baseline (28 weeks) and after treatment (at 40 weeks)

| | LETO (n = 5) | OLETF (n = 20) | | | |
|---|---|---|---|---|---|
| | | Control (n = 5) | Los (n = 5) | Ros (n = 5) | Los + Ros (n = 5) |
| **At 28 weeks** | | | | | |
| LVEDD (mm) | 7.6 ± 0.2 | 8.0 ± 0.5 | 7.9 ± 0.4 | 8.0 ± 0.5 | 8.1 ± 0.6 |
| LVESD (mm) | 5.6 ± 0.4 | 5.8 ± 0.5 | 5.7 ± 0.4 | 5.8 ± 0.6 | 5.9 ± 0.4 |
| FS (%) | 26.4 ± 2.1 | 24.0 ± 2.8 | 23.2± 3.2 | 23.6 ± 2.9 | 23.6 ± 1.9 |
| LVEF (%) | 60.1 ± 4.3 | 58.7 ± 5.0 | 62.8 ± 4.8 | 61.0 ± 5.4 | 58.5 ± 4.2 |
| Global radial strain (%) | 60.1 ± 5.8 | 52.8 ± 8.1* | 50.2 ± 5.5* | 49.8 ± 6.1* | 53.7 ± 8.0 * |
| Global radial strain rate | 9.7 ± 1.2 | 8.5 ± 2.1* | 8.8 ± 3.6* | 8.9 ± 1.9* | 8.3 ± 2.6* |
| **At 40 weeks** | | | | | |
| LVEDD (mm) | 7.7 ± 0.3 | 8.2 ± 0.5 * | 7.5 ± 0.4 † | 7.7 ± 0.3 † | 7.5 ± 0.5 † |
| LVESD (mm) | 5.7 ± 0.5 | 5.9 ± 0.3 | 5.4 ± 0.4 † | 6.3 ± 0.6 † | 5.0 ± 0.6 † |
| FS (%) | 25.6 ± 2.6 | 23.2 ± 2.7 * | 28.2 ± 2.3* † | 24.9 ± 5.1 | 30.3 ± 4.8*† |
| LVEF (%) | 58.1 ± 4.0 | 55.3 ± 5.9 | 60.3 ± 3.9 † | 54.0 ± 7.4* | 61.6 ± 5.2 † |
| Global radial strain (%) | 55.5 ±7.0 | 44.3± 10.5* | 59.3 ± 6.7 † | 49.8 ± 6.0* | 55.7 ± 4.5 † |
| Global radial strain rate | 8.5 ± 1.9 | 7.8 ± 2.7 * | 8.8 ± 2.6 † | 7.9± 1.5 * | 8.5 ± 2.1 † |

LOS, losartan; ROS; rosiglitazone; LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension; LVEF, left ventricular ejection fraction

* $p$ <0.05, compared with the untreated LETO rats.

† $p$ <0.05, compared with the untreated OLETF rats.

## IV. DISCUSSION

The results of the present study demonstrate the followings. (1) the effect of rosiglitazone (a PPAR-r agonist) and losartan (an ARB) on myocardial function as well as structural change of myocardium; (2) myocardial changes and the association with the degree of microalbuminuria in the type 2 diabetic rat model.

The development of myocardial dysfunction is a major complication of diabetes mellitus. This condition is characterized by defects of contractile and relaxation function in the absence of significant coronary artery disease or systemic hypertension. In the past 3 decades, a number of experimental, pathological, epidemiological, and clinical data were reported that confirmed the association of diabetes with myocardial dysfunction. The pathogenesis of myocardial dysfunction in diabetes remains unclear, although several mechanisms, including metabolic disturbances, myocardial fibrosis, microangiopathy, autonomic dysfunction, and insulin resistancem have been proposed. In these mechanisms, the alteration of microvascular structure and function is considered to be an important mechanism.

Albuminuria has been considered to be a marker of a generalized vascular dysfunction. It has been shown to predict cardiovascular morbidity and mortality in diabetic patients independent of conventional cardiovascular risk factors including age, arterial hypertension, and hypercholesterolemia. Although the mechanism of the association of albuminuria with cardiac events is not clear, it is possible that the vascular changes leading to renal dysfunction may also be present in the vasculature of the heart and thus

contribute to cardiac dysfunction. In diabetic hearts, morphological changes of small vessels characterized by microangiopathy were seen in several animal and autopsy studies. Through a prior clinical study, we demonstrated that Doppler strain and strain rate imaging detected subclinical left ventricular systolic and diastolic dysfunction in diabetic patients with albuminuria. In addition, it was proven that albuminuria was associated with myocardial dysfunction in diabetic patients without overt heart disease.

Angiotensin II plays a key pathophysiological role in the progression of diabetic renal disease, and blockade of the renin–angiotensin system with ACE inhibitors or ARBs has therefore become an important therapeutic strategy to reduce renal and cardiovascular events in patients with diabetes. Yasuda et al demonstrated that Losartan affects the reduction of albuminuria and the progression of renal disease from microalbuminuria to macroalbuminuria.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcription factors that have been shown to play important roles in maintaining glucose and lipid homeostasis. Thiazolidinediones may modify the risk of vascular complications in diabetes, raise high-density lipoprotein cholesterol levels, and lower triglycerides. It was reported that PPARα and γ agonist attenuate diabetic kidney disease in the apolipoprotein E knockout mouse and rosiglitazone improves both plasma glucose and blood pressure levels. The beneficial effects of thiazolidinediones on hyperglycemia and cardiovascular risk factors have made them attractive agents in patients with type 2 diabetes. However, in clinical practice, edema can occur in patients

treated with rosiglitazone and sometimes produce signs of heart failure as a side effect although a few animcal experimental studies demonstrated the beneficial effects of thiazolidinediones of reducing left ventricular collagen accumulation and improving left ventricular function. In our results. Rosiglitazone couldn't prevent the progression of myocardial dysfunction. However, in combination with losartan, the protective effects were enhanced compared with treatment of each drug.

Limitations of this study need to be addressed. First, the hemodynamic parameters such as blood pressure and left ventricular pressure were not evaluated, so we cannot completely exclude the adverse effects of high blood pressure on the diabetic myocardium. Second, this experimental study was performed with a small number of LETO and OLETF rats.

## V. CONCLUSION

The ARB, losartan revealed protective effect on the progression of microalbuminuria, myocardial structural and functional changes. The PPAR-r agonist, rosiglitazone showed minimal protective effect on the progression of microalbuminuria and no significant myocardial structural and functional benefits even though it had a metabolic benefits. The combination of losartan and rosiglitazone showed both metabolic benefits and myocardial protective effects.

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< ABSTRACT(IN KOREAN)>

# 제 2형 당뇨 OLETF 백서에서 심근 구조와 기능에 대한 PPAR-γ agonist인 rosiglitazone과 angiotensin II 수용체 억제제인 losartan의 효과

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당뇨병 환자에서 알부민뇨가 동반되는 경우에 심근 기능이상이 종종 관찰된다. 본 연구의 목적은 제 2형 당뇨 백서모델에서 심근의 구조와 기능에 peroxisome proliferator-activated receptor (PPAR) ligand와 angiotensin II 수용체 억제제의 효과를 규명하고 각 약제의 효과와 알부민 뇨의 연관성을 확인하고자 하였다. 5 마리의 Long-Evans Tokushima Otsuka (LETO) 백서와 20마리의 Otsuka Long-Evans Tokushima Fatty (OLETF) 백서를 연구에 이용하였으며, OLETF 백서를 28주령에 무작위로 5마리씩, 네 그룹으로 분류하였다. Group I = 약제를 투여하지 않는 LETO 백서; Group II = 약제를 투여하지 않는 OLETF 백서; Group III = losartan을 투여하는 OLETF 백서; Group IV = rosiglitazone을 투여하는 OLETF 백서; Group V = losartan과 rosiglitazone을 함께 투여하는 OLETF 백서였다. Angiotensin II 수용체 억제제인 losartan은 5 mg/kg/d의 용량으로, PPAR-gamma agonist인 rosiglitazone은 3 mg/kg/d의 용량으로 먹이에 섞어서 12주간 투여하였다.

소변과 혈액샘플은 28주령과 치료가 끝난 40주령에 모았으며, 이면성 심초음파와 strain rate 영상 또한 심장의 구조와 기능을 평가하기 위해서

28주령과 40주령에 얻었다. 실험기간이 모두 종료되는 40주령에 심장을 분리하여 조직학적 분석을 시행하였다.

OLETF 백서는 LETO 백서에 비해서 28주령과 40주령 모두에서 보다 높은 공복혈당 수치, 공복 인슐린 수치와 높은 소변 알부민대 크레아티닌 비율을 보였다. 40주령에 rosiglitaxone을 투여하였거나 두가지 약제를 함께 투여한 경우에 rosiglitazone이 투여되지 않은 그룹보다 좋은 대사 수치를 나타냈다. 미세알부민뇨의 관점에서는 losartan을 투여하였거나 두가지 약제를 함께 투여한 경우 미세알부민뇨의 진행이 의미있게 약해졌다. TGF-β나 TNF-α 와 같은 성장인자와 IL-1과 IL-6와 같은 염증 활성인자 또한 LETO 백서에 비해서 OLETF 백서의 경우 의미있게 증가되어 있음을 확인할 수 있었다. Losartan이나 병합요법으로 치료를 한 후에는 이들 인자들의 발현은 LETO 심장에서의 발현정도와 거의 비슷해졌다. 심장의 구조와 기능의 분석 결과, 간질 섬유화와 콜라젠 침착과 같은 조직학적 결과 또한 losartan 투여 또는 병합 투여시에 감소하였고 rosiglitazone 만 투여한 경우는 호전이 없었다. 이와 같은 결과는 이면성 심초음파와 strain rate 영상 분석결과에서도 비슷하게 나타났다. 결론적으로 angiotension II 수용체 억제제인 losartan은 알부민뇨의 진행 및 심근 구조와 기능의 변화에 보호작용이 있었으며 PPAR-γ agonist이 rosiglitazone은 알부민뇨의 진행에는 약한 보호작용을 보이고, 대사적 이득 또한 있었으나, 심근의 구조와 기능에는 보호효과가 없었다. Losartan과 rosiglitazone의 병합시에는 대사적인 이득과 심근의 보호효과를 모두 얻을 수 있었다.

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핵심되는 말 : angiotensin II 수용체 차단제, peroxisome proliferator-activated receptor- γ agonist, 당뇨, 알부민뇨, 심근