Effects of spironolactone, losartan and combination therapy on diabetic nephropathy in OLETF rats

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And I would like to give great thanks and love for my family members, Mom, you'll be the winner!

Thank You

12th July, the 8th Birthday of my loving daughter.

Myoung Sook Shim

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Abstract

Effects of spironolactone, losartan and combination therapy on diabetic nephropathy in OLETF rats

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Backgrounds **Although there were some evidences that spironolactone could attenuate albuminuria in type 2 diabetes via anti-inflammatory and anti-oxidants effects, the effects of spironolactone on VEGF expression on kidney in diabetic nephropathy were not elucidated. In this study, we examined the effects of spironolactone, losartan, and combination with spironolactone and losartan on albuminuria and glomerular VEGF expression in type 2 diabetic rat model.**

Methods **Thirty-three OLETF rats were divided into the following four groups and treated with different medications from 25 weeks to 50 weeks:**

control OLETF group for diabetic controls (N=5), spironolactone group (N=10), losartan group (N=9), and combination group (N=9). At 15, 30, and 50 weeks, urine was collected for 24 hours urine protein amounts and albumin-creatinine-ratio (ACR). At 50 weeks, all experimental rats were sacrificed and both kidneys were prepared for western blot and RT-PCR for VEGF, TGF-β, and type IV collagen.

Results **At 50 weeks, ACR was significantly decreased in losartan and combination regimen treated group (1.21±0.81, 1.01±0.99, $p<0.05$) compared with that of control OLETF group (4.35±1.19). But, in spironolactone treated group, ACR was not decreased. There was a significant reduction in glomerular VEGF mRNA levels in spironolactone and combination regimen treated group compared with control OLETF group. But, western blot did not show significant difference among groups. TGF-β and type IV collagen expressions were significantly decreased in spironolactone and combination regimen treated groups. MDA levels were significantly decreased in combination regimen treated group than that of control diabetic rat group.**

Conclusion **These results suggest that combination therapy of spironolactone and losartan may contribute the beneficial effect on diabetic**

nephropathy by reducing VEGF, TGF - β, type IV collagen expression and oxidative stress in type 2 diabetic rat models.

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Key Words: **Spironolactone, losartan, diabetic nephropathy, vascular endothelial growth factor**

I. Introduction

It is well known that renin-angiotensin-aldosterone system (RAA system) has an important role in cardiovascular disease, diabetic nephropathy, and chronic renal disease through a mechanism of inflammation, fibrosis, and necrosis[1-5]. For these reasons, the angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) are established as an effective regimen for chronic heart failure and diabetic nephropathy [6-11].

In the long term use of renin angiotensin system blockade, plasma aldosterone levels have been shown to increase in 40% of patients with diabetic nephropathy and 20% of patients with chronic renal failure which is called aldosterone escape phenomenon [12-13]. In these patients, proteinuria-reducing effects of ACEI and ARB are also decreased [14]. In these aspects, a reduction of aldosterone level itself is thought to be an important indicator of treatment.

Recent data showed that aldosterone receptor blocker could reduce proteinuria via decreasing various growth factors [15-18]. Also, it was reported that aldosterone receptor blocker treatment has an additional effect on reduction of proteinuria in patients with chronic renal disease who were failed to a treatment of ACEI [19].

Vascular endothelial growth factor (VEGF), one of the strong

angiogenetic factors, has been known to have important role in neovascularization of atherosclerotic plaque or solid cancers [20-22] and also progression of diabetic nephropathy [23]. Although it is reported that ARB treatment has protective effects on diabetic nephropathy by reducing VEGF [24, 25], there is no data about the effect aldosterone receptor blocker on renal VEGF expression.

In this study, we investigated the effects of losartan, spironolactone and their combination treatment on proteinuria and renal VEGF expression in type 2 diabetic rat model.

II. Materials and Methods

1. Animals and drugs

Thirty-three male Otsuka-Long-Evans-Tokushima-Fatty (OLETF, Otsuka Pharmaceutical, Tokushima, Japan) rats were used. They were divided into 4 groups at the age of 25 weeks and received spironolactone (50mg/kg/day) and losartan (20 mg/kg/day) in drinking water until 50 weeks. All rats were given free access to standard rat food and drinking water.

First is diabetic control OLETF group (CO, N=5), second is spironolactone treated diabetic group (SPR, N=10), third is losartan treated diabetic group (LO, N=9) and fourth is spironolactone and losartan combination treated diabetic group (COM, N=10). The research protocol was approved by the animal ethics committee of the Yonsei University Wonju College of Medicine (Wonju, Korea).

2. Basal parameters

At the age of 15, 30, 40, 50 weeks, body weights and blood glucose (Surestep^{R}, Lifescan Inc, MA, USA) were checked. Blood pressure was examined by using tail-cuff plethysmography at 30, 40, 50 weeks. Twenty four hour urine was collected at 15, 25, 30, 50 weeks for measuring urine protein amounts (Roche Molecular Biochemicals, Indianapolis, IN) and

albumin-creatinine ratio (ACR) by ELISA (Shibayagi, Shibukawa, Japan) method.

3. Kidney extraction

At 50 weeks, all experimental rats were sacrificed under anesthesia by intraperitoneal injection of ketamine (70 mg/kg). One kidney was preserved using a quick freeze method with liquid nitrogen while the contralateral kidney was fixed in 4% paraformaldehyde for 48 hours, and then embedded in paraffin for histological examination and immunohistochemical staining of VEGF. Right kidneys were rapidly frozen by liquid nitrogen and stored at -70℃ for western blotting and real time RT PCR.

4. Kidney histologic examination

Paraffin-embedded kidney tissues were cut into 7 μm-thick slices and stained with periodic acid-Schiff (PAS). Glomerular matrix index (GMI) score was measured for glomerulosclerosis. The severity of sclerosis for each glomerulus was graded from 0 to 4 as follows [26]: Grade 0, normal; grade 1, mild sclerosis (less than 25% of glomerulus); grade 2, moderate sclerosis (25~50% of glomerulus); grade 3, moderate-severe sclerosis (50-75% of glomerulus); grade 4, severe sclerosis (75~100% of glomerulus) and for each rat no less than 20 glomeruli were analyzed in each kidney section.

5. Immunohistochemical stain of VEGF

The kidney tissues were fixed in 4% paraformaldehyde and subsequently embedded in paraffin. Serial 7 μm-thick sections were obtained and fixed on the slides. The sections were deparaffinized for immunohistochemical stain. Then slides were transferred to 10-mmol/L citrated buffer solution (pH 6.0) and washed with distilled water and then 0.05% H_2O_2-methanol was applied for 15 min, after that added 1st antigen 1:1000 monoclonal anti-VEGF antibody (Santa Cruz Biotechnology Inc., SC, USA) at room temperature. Next, biotinylated secondary antibody in rat ABC staining system (Santa Cruz Biotechnology Inc., SC, USA) was added, and then avidin and biotinylated horseradish peroxidase (ABC reagents) are also added. At last, the slides were incubated in peroxidase substrates containing 0.05% 3, 3′-diaminobenzidine tetrahydrochloride (DAB). Stained tissues were observed under light microscope with charged-coupled devices camera (Pulnix, MA, USA), glomerular images were sent to computer monitor and then VEGF optical densities were measured by image analyzer.

6. Real time RT-PCR

Total RNA was extracted from the kidney tissues which stored at -70℃ by snap-frozen in liquid nitrogen with TRIzol LS reagent (GIBCO BRL, USA) and total RNA was reverse transcribed into cDNA using oligo-(dT)

primer (Promega, Madison, WI, USA).

The real-time RT-PCR was performed by using SYBR Green RT-PCR kit (Qiagen, Valencia, CA) and measured with Roter-Gene RG-3000 cycler (Corbett Research, Mortlake, NSW, Australia). Primer oligonucleotide sequences for VEGF, TGF-β, collagen type IV and GAPDH are as follows.

VEGF forward; 5' -GTATATCTTCAAGCCGTCCTGTGTG-3'

VEGF reverse; 5' -GATCCGCATGATCTGCATAGTGAC-3'

TGF-β forward; 5' -TGAGTGGCTGTCTTTTGACG-3'

TGF-β reverse; 5' -TGGGACTGATCCCATTGATT-3'

Collagen type IV forward; 5' -CCAGGATTCCAAGGTCAGAA-3'

Collagen type IV reverse; 5' -CCCTGGTTCTCCTTTGATGA-3'

GAPDH forward; 5' -TCAGGTCATCACTATCGGCAATG-3'

GAPDH reverse; GGAATTGAATGTAGTTTCATGGATGC-3'

The real-time RT-PCR was performed by running for 10 min at 95℃, subsequently, 40 cycles that consisting of denaturation for 15s at 94℃, annealing for 30s at 58℃ and extension 30s at 72℃ were applied. After that rinse and melting process were applied, detection of fluorescent products was done at 92℃ extension. The cycle threshold ($\triangle C_t = C_{t\ VEGF} - C_{t\ \beta\text{-actin}}$) of each samples were calculated and the relative change ratio was calculated using VEGF/β-actin mRNA ratio.

7. Western blot analysis

The cortex of each kidney was homogenized in RIPA buffer and the lysates were incubated in ice for 20 min and then centrifuged at 15,000 rpm to remove cellular debris. 10 μg of proteins were electrophoresesed on 10% SDS-PAGE gels at 100V. Proteins were transferred to Polyvinylidene fluoride PVDF membrane for 1h at 280mA in Tris-based buffer. Non-specific binding sites were blocked with 5% non-fat dried milk for 1h and membranes incubated anti-rat beta actin (1:2000 dilution; Cell signaling, USA) or VEGF antibody (1:1000 dilution; R&D System, Minneapolis, USA) for overnight. Antibody-treated membranes were visualized by the ELC Western Blotting Analysis System (Amersham Biosicences, Buckinghamshire, UK) with anti-rabbit IgG or anti-goat IgG-HRP antibody.

8. MCP-1 and MDA

MCP-1 levels as inflammatory marker were measured in 24 hours urine by quantitative sandwich ELISA kit (Biosource Inc., CA, USA) [29] and MDA(Malonyldialdehyde) as oxidative stress marker were measured by fluorometric HPLC method.

9. Statistical analysis

All data are presented as means ± SD. Oneway ANOVA, Turkey test (multiple comparisons) windows SPSS 12.0 was used for statistical analysis. Western blot results were revised by beta actin then used Kruskall-Wallis test and Mann-Whitney test. *P*< 0.05 was considered to be statistically significant.

II. Results

1. Changes of body weight, blood glucose and blood pressure

There were no differences in body weights and plasma glucose levels at each age. But, blood pressure levels of LO and COM group at 40 weeks, and COM group at 50 weeks were significantly lower than that of CO group (Table 1).

Table 1. Characteristics of experimental rats

	15 wks	30 wks	40 wks	50 wks
Body weights (g)				
CO	554.60±105.06	608.00±45.49	574.00±25.09	602.00±46.04
SPR	489.30±40.27	589.90±74.11	585.70±76.49	596.00±80.71
LO	450.11±32.67*	554.56±65.45	558.11±80.21	580.56±97.45
COM	465.11±68.46	570.78±86.22	562.33±66.40	577.22±78.54
Blood glucose (mg/dL)				
CO	113.82±17.02	140.20±17.41	123.20±19.86	140.80±20.29
SPR	118.60±17.01	137.60±18.07	122.70±16.58	146.60±35.30
LO	107.56±12.69	144.00±21.14	113.89±13.10	123.67±16.90
COM	119.89±18.08	136.67±12.67	123.89±18.63*	127.69±16.88
Blood pressure (mmHg)				
CO		117.60±4.75	135.94±9.17	132.11±10.26
SPR		116.40±9.01	123.79±5.85	132.11±13.23
LO		123.19±6.19	116.76±12.20*	131.02±8.19
COM		117.38±6.71	115.61±12.74*	109.30±10.24*

Data are represented by mean ± SD. *: $p<0.05$ compared with CO group.

CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group.

2. 24 hours urine protein amounts and albumin-creatinine ratio (ACR)

At 15 and 30 weeks, there were no differences in 24 hours urine protein amounts and ACR among all groups. At 50 weeks, LO and COM groups showed significant decrease in 24 hours urine protein amounts and ACR, but SPR group could not show the reduction of proteinuria and ACR compared with CO group (Table 2).

Table 2. Changes in ACR and 24-hour urine protein amounts

	15 wks	30 wks	50 wks
ACR (mg/mgCr)			
CO	0.50 ± 0.13	1.01 ± 0.96	4.35 ± 1.19
SPR	0.39 ± 0.31	0.93 ± 0.62	3.26 ± 1.74
LO	0.26 ± 0.14	0.82 ± 0.81	1.21 ± 0.81*
COM	0.43 ± 0.11	0.96 ± 0.95	1.06 ± 0.98*
24 hr urine protein levels (mg/day)			
CO	4.67 ± 1.26	34.47 ± 12.22	77.04 ± 23.57
SPR	11.29 ± 7.68	30.64 ± 28.42	70.24 ± 38.84
LO	10.49 ± 4.71	27.91 ± 23.99	29.23 ± 22.14*
COM	9.61 ± 4.03	26.15 ± 21.90	21.77 ± 11.34*

Data are represented by mean ± SD. *: $p<0.05$ compared with CO group.

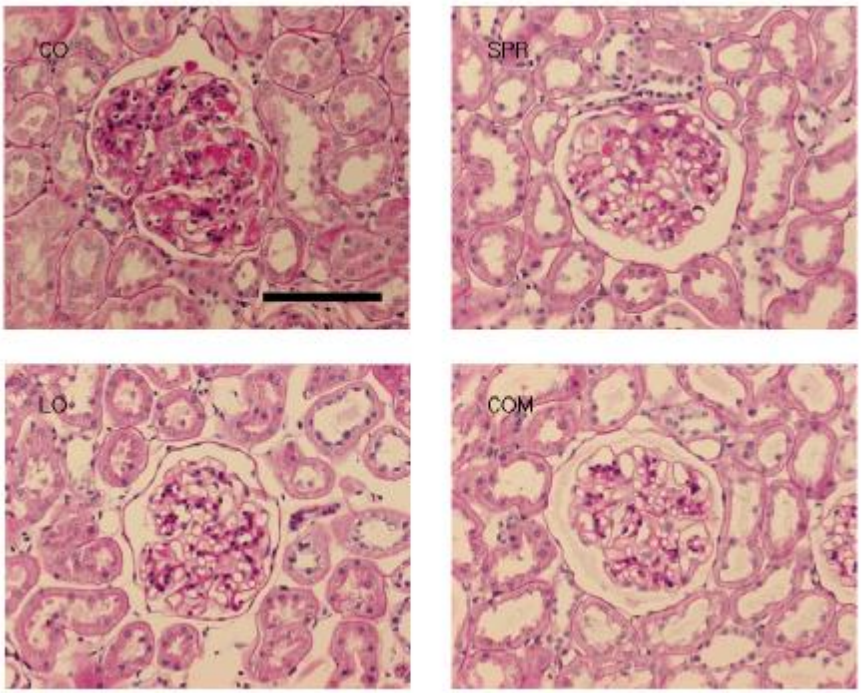
CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group

3. PAS staining of glomeruli and glomerular matrix index (GMI) score

Glomerular mesangial expansion was observed in CO group compared with other groups in PAS staining of glomeruli (Fig. 1 - A).

Glomerular matrix index (GMI) scores were significantly decreased in all medication treatment groups compared with CO group. Also, COM group showed marked decrement of GMI scores than those of SPR and LO groups (Fig. 1 - B).

A.



B.

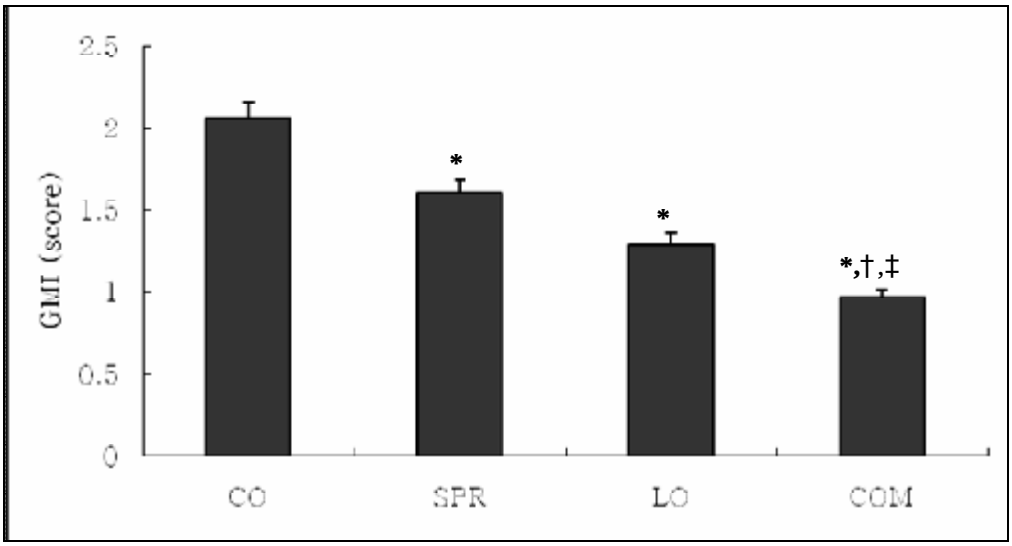
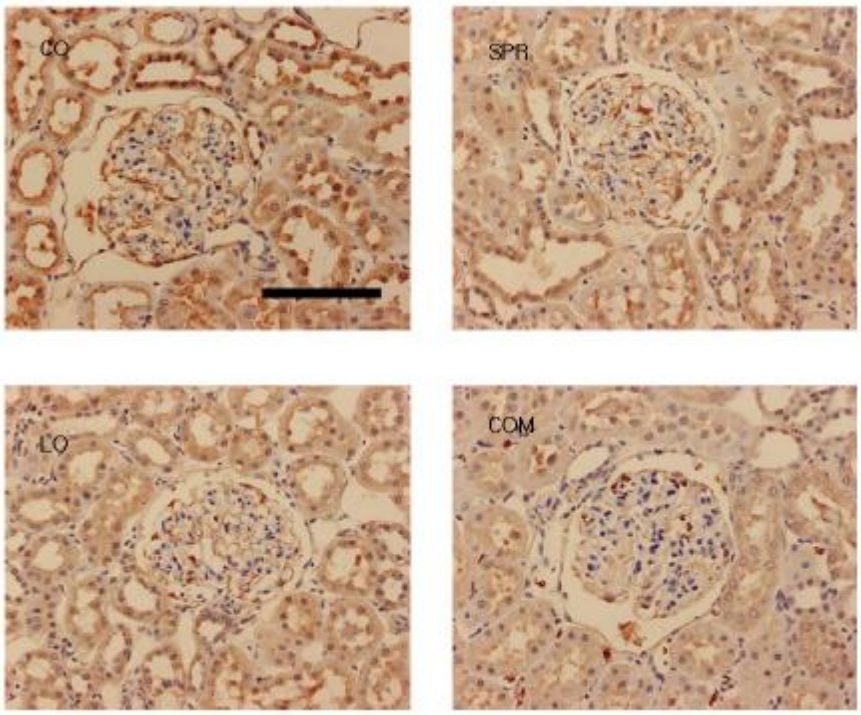


Figure 1. PAS staining of glomeruli (A) and glomerular matrix indices (GMI) (B). PAS staining of glomeruli showed marked mesangial expansion and sclerosis in CO group compared with other groups. Compared with CO group, GMI scores were significantly decreased in SPR, LO, and COM groups. CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group. *: *p*<0.05 compared with CO group. †: p<0.05 compared with SPR group. ‡: p<0.05 compared with LO group. Scale bar, 100 ㎛.

4. Immunohistochemical staining for VEGF

Immunohistochemical staining for glomerular VEGF revealed a clear increase in VEGF expression in the CO group compared with all medication treated groups (Fig. 2-A). The optical density of immunohistochemical staining for VEGF in the LO and COM groups were significantly increased compared with CO group. But SPR group showed no difference compared with CO group (Fig. 2-B).

A.



B.

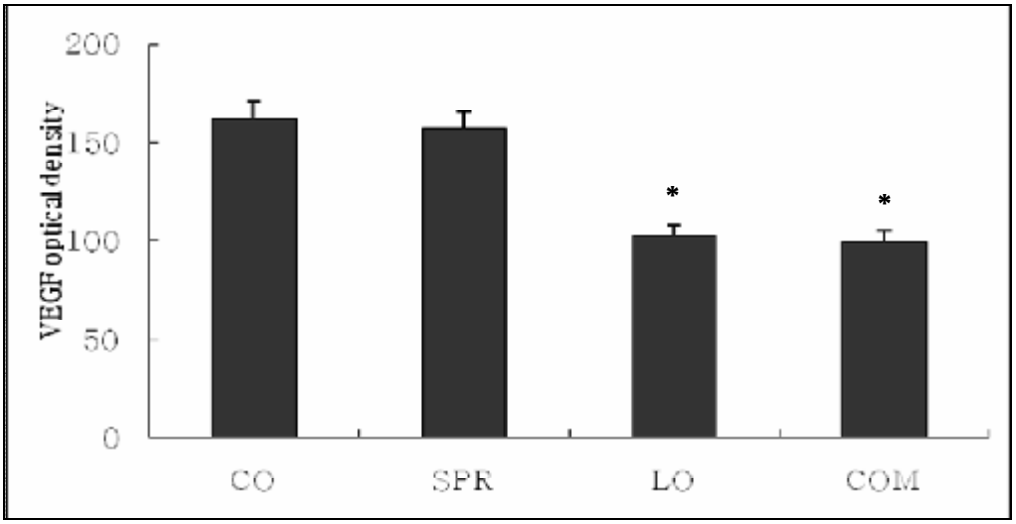


Figure 2. Immunohistochemical staining (A) and optical densities (B) for glomerular VEGF. Compared with LO and COM groups, darker pigmentation of glomeruli was shown in CO and SPR group. Optical densities of glomerular VEGF in LO and COM groups were significantly lower than those of CO and SPR groups. CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group. *: $p<0.05$ compared with CO group. Scale bar, 100 ㎛.

5. Real time RT - PCR for VEGF, TGF - β, type IV collagen

Quantitative analysis revealed that VEGF mRNA expression was 0.61 - fold at SPR group, 0.47 - fold at LO group, and 0.63 - fold at COM group when compared with CO group (Fig. 3). TGF - β mRNA expression was significantly decreased in all treatment groups compared to CO group. Also, SPR and COM groups showed lower expression of TGF - β mRNA than that of LO group (Fig. 4). Type IV Collagen mRNA expression was significantly decreased in SPR and COM groups compared with CO group (Fig. 4).

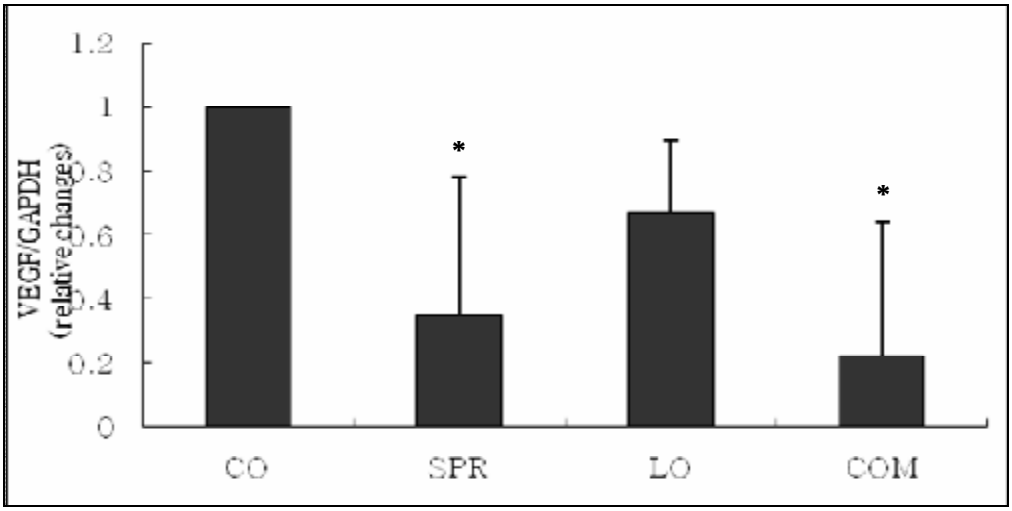


Figure 3. VEGF mRNA expression by real-time RT-PCR. Quantitative analysis revealed that expression of VEGF mRNA was 0.61-fold at SPR group, 0.47-fold at LO group, and 0.63-fold at COM group when compared with CO group. CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group. *: $p<0.05$ compared with CO group.

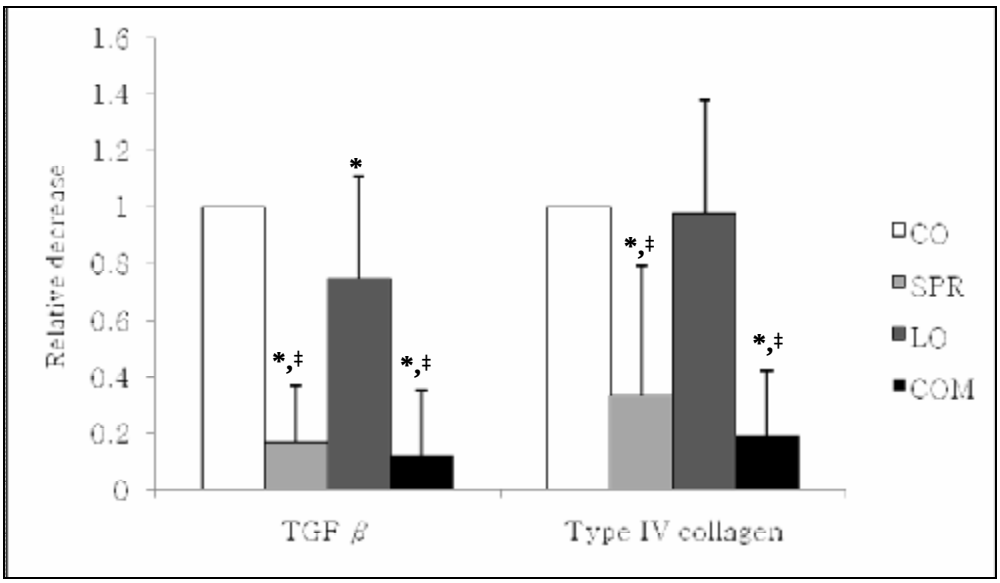


Figure 4. TGF - β and collagen type IV mRNA expression by real - time RT - PCR. Compared with CO group, TGF - β and collagen type IV mRNA expression of SPR and COM groups were significantly decreased. CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group. *: $p<0.05$ compared with CO group, †: $p<0.05$ compared with LO group.

6. Western blot of VEGF protein expression

Western blot did not show any differences among groups (Fig.5).

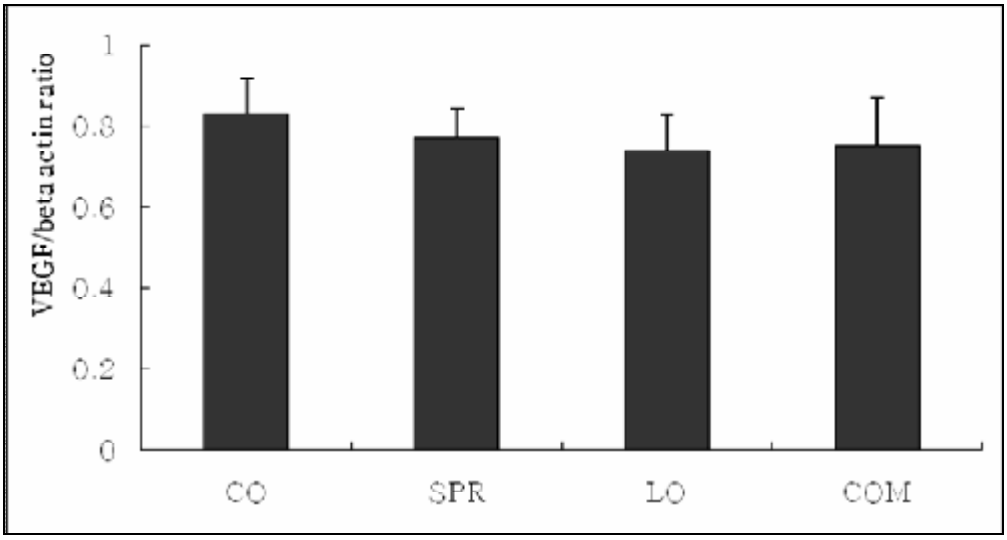


Figure 5. Western blot for renal VEGF expression. Western blot did not show any differences among groups.

7. MCP - 1 and MDA

At 15 and 50 weeks, 24 hours MCP - 1 levels were not different among four groups just showing decreasing tendency in COM group (Fig. 6). At 50 weeks, MDA levels were significantly decreased in COM group compared with CO group (Fig. 7).

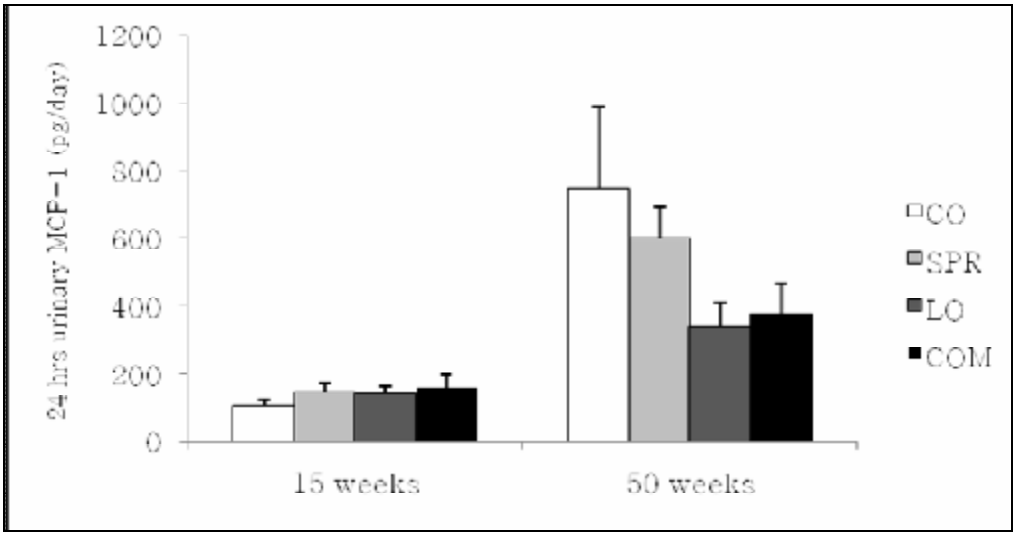


Figure 6. 24 hours urinary MCP - 1 levels. MCP - 1 levels were not different among four groups just showing decreasing tendency in COM group. CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group.

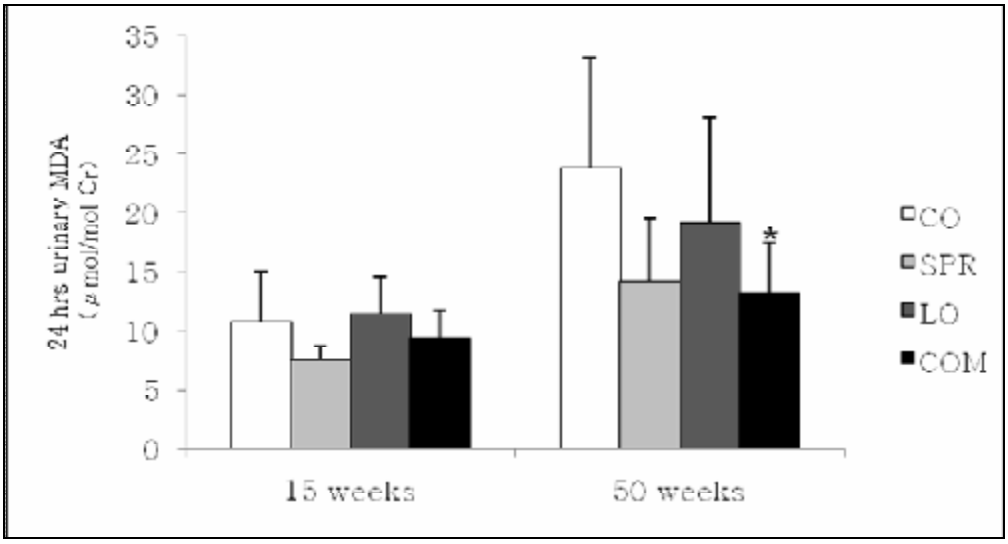


Figure 7. 24 hours urinary MDA levels. MDA levels were significantly decreased in COM group than that of CO group. CO, control OLETF group; SPR, spironolactone treated OLETF group; LO, losartan treated OLETF group; COM, spironolactone and losartan treated OLETF group. *: $p<0.05$ compared with CO group.

IV. Discussion

We investigated the effects of a combination treatment consisting of losartan (angiotensin receptor blocker, ARB) and spironolactone (aldosterone receptor blocker) for proteinuria and renal VEGF expression in a type 2 diabetic rat model.

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) and diabetic ESRD patients are more prone to cardiovascular mortality than other caused ESRD patients. Therefore, early identification and renoprotective treatment are critical for the prevention of end organ damage from diabetic nephropathy [27-28].

It is well known that treatments with angiontensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) could retard the progression of diabetic nephropathy, chronic heart failure and chronic renal failure through the reduction of blood pressure and anti-inflammatory, anti-sclerosing effects [6-11]. Recent reports indicate that spironolactone, an aldosterone receptor blocker, reduces proteinuria by decreasing oxidative stress and providing anti-inflammatory effects [16-18, 29, 30]. Although aldosterone receptor blockers may be useful for the treatment of diabetic nephropathy, they could be restrictively used due to hyperkalemia. Saito et al and Sepkens et al reported that hyperkalemia exceeding 5.5mEq/L may occurred even if the dose of spironolactone is as low as 25 mg. Thus it is essential to monitor serum potassium [31-32].

In our data, losartan and combination regimen treated rat groups showed significant decreases in 24 hours urine protein amounts and ACR. But, spironolactone monotherapy group did not show the significant reduction in 24 hours urine protein amounts and ACR. This data may be explained by the difference of treatment period of spironolactone. Recent studies reported that treatment with aldosterone receptor blockers in the early stages of nephropathy may reduce proteinuria, but it has no beneficial effects at later stages [33-34]. Because we started the spironolactone treatment at 25 weeks, effects of spironolactone on diabetic nephropathy could be underestimated compared with other studies that initiated the treatement at early stage [16]. Another possible explanation is the different effects of spironolacone between hemodynamics and fibrosis process. PAS staining of glomeruli revealed marked mesangial expansion and sclerosis in the diabetic control group compared to other groups. Real time RT-PCR for TGF-beta and collagen IV, which are well known factors of renal fibrosis, showed decreased levels in the spironolactone treated group and in the spironolactone and losartan combination group in agreement with the results of previous studies [35-36]. But, there were no differences in blood pressure between the spironolactone treated group and other groups. That might mean that spironolactone could operate the action through non-hemodynamic mechanism. This phenomenon might imply that proteinuria were resulted from different mechanisms from renal fibrosis. Also, spironolactone might effects on renal fibrosis but could not effect on

hemodynamics in our study [37].

In diabetic nephropathy, the VEGF expression may be increased by various growth factors including platelet derived growth factor (PDGF) and accumulation of hyperglycemia-induced advanced glycated end products (AGEs) [38-41]. Recent studies revealed that ACEI and ARB could be decrease the proteinuria by reducing VEGF expression in type 2 diabetic rat model [24, 42]. In the present study, we compared the glomerular VEGF mRNA expression after treatment with losartan, spironolactone, and a combination therapy of losartan and spironolactone in a type 2 diabetes rat model. The spironolactone treated group and combination group exhibited marked reduction of VEGF mRNA expression compared to the diabetic control group. The immunohistochemical expression of VEGF showed marked decreases in the losartan treated group and in the combination group, but the spironolactone treated group was not significantly different from the diabetic control group. There was a tendency toward decreased VEGF expression in the combination treatment group according to the results of western blot analysis, but this difference was not statistically significant. These data could partially suggested that combination therapy with losartan and spironolactone may effect on diabetic nephropathy by reducing VEGF expression. But, the discrepancies between the results of mRNA expression and protein levels may be a limitation of the interpretation of our data. This difference may be explained that analyses for VEGF may be due to varying tissue components. Besides we examined only glomeruli by immunohistochemical staining, we

could not isolate glomeruli from with tubules and interstitial tissue in mRNA and Western blot analysis.

Activation of the tissue renin-angiotensin system (RAS) causes increased production of reactive oxygen species (ROS) through activation of the NADPH oxidase enzymatic complex in numerous tissues, including renal tissue. Angiotensin and other cytokines can cause diabetic nephropathy through inflammatory mechanisms [43-44]. Blockade of the RAS may have protective effects against diabetic nephropathy through anti-oxidative and anti-inflammatory mechanisms [29-30]. In our study, only a combination therapy of losartan and spironolactone reduced levels of the malonyldialdehyde (MDA)-oxidative stress marker. MCP-1, an inflammatory marker, also tended to decrease only in rats treated with both therapies. These results may indirectly explain that spironolactone and losartan combination therapy could be helpful for the treatment of diabetic nephropathy.

V. Conclusion

In conclusion, we suggested that a combination treatment including both angiotensin receptor blocker and aldosterone receptor blocker may decrease proteinuria in patients with diabetic nephropathy by reducing VEGF expression, TGF-β and type IV collagen expression. Also, we proposed that combination treatment may be beneficial for diabetic nephropathy through anti-inflammatory and anti-oxidative mechanisms.

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국문요약

Effects of spironolactone, losartan and combination therapy on diabetic nephropathy in OLETF rats

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연구배경: 현재까지 알도스테론수용체억제제가 제**2**형 당뇨병에서 항염증작용 및 항산화작용을 통하여 알부민뇨를 감소시켰다는 보고는 있으나 혈관내피성장인자에 대한 영향에 대해서는 아직 밝혀지지 않은 상태이다. 본 연구에서는 제**2**형 당뇨병 쥐 모델에서 안지오텐신수용체억제제인 **losartan** 또는 알도스테론수용체억제제인 **spironolactone**을 처리하였을 경우 또한 이들 약제를 동시에 투여하였을 경우 혈관내피성장인자를 억제하여 알부민뇨를 감소시킬 수 있는지 알아보고자 하였다.

방법: **33**마리의 **OLETF**쥐를 생후 **5**주에 분양 받아 **25**주부터 **50**주까지 **4**군으로 나누어 실험하였다. 각군은 당뇨병 대조군인 **CO**군 **(N=5), spironolactone** 처치 당뇨병군, **SPR**군**(N=10), losartan** 처치 당뇨병군, **LO**군**(N=9),** 그리고 **spironolactone**과 **losartan**을 동시에 투여한 당뇨병군, **COM**군**(N=9)**으로 구성하여 실험을 진행하였다. 생후 **15**주, **25**주, **30**주, **50**주에 **24**시간 소변을 모아

알부민-크레아티닌 비와 **24**시간 소변 단백량을 측정하였다. 생후 **50**주에 실험쥐들을 희생시킨 후 양측 신장을 보관하여 **VEGF, TGF-β, type IV collagen**의 **mRNA** 측정 및 **western blot**을 시행하였다.

결과: 생후 **50**주에 비교한 **24**시간 소변 단백량 및 알부민-크레아티닌 비는 **CO**군에 비하여 **LO**군이나 **COM**군에서 현저하게 감소되었으나 **SPR**군에서는 **CO**군과 차이가 없었다. 신장사구체에서 **VEGF mRNA** 발현은 **CO**군에 비하여 **SPR**군과 **COM**군에서 의미 있게 감소하였다. **Western blot**에서는 **COM**군에서만 감소하는 양상을 보였으나 통계적으로는 차이가 없었다. **VEGF**에 대한 면역조직화학염색 결과 **CO**군에 비하여 **LO**군과 **COM**군에서는 감소하였지만 **SPR**군에서는 큰 차이를 보이지 않았다. **TGF-β**와 **type IV collagen**의 발현은 **SPR**군과 **COM**군에서 의미 있게 감소하였고 **MDA**는 **COM**군에서만 의미 있게 감소하였다.

결론: 당뇨병성 신증 치료에 있어 알도스테론수용체억제제와 안지오텐신수용체억제제를 동시에 투여하는 것은 **VEGF** 발현 및 **TGF-β, type IV collagen,** 산화 스트레스를 감소시켜 당뇨병선 신증의 치료에 효과가 있을 것으로 생각된다.

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중심 단어: 알도스테론수용체억제제, 안지오텐신수용체억제제, 당뇨병성 신증, 혈관내피성장인자