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The effect of cyclosporine on P-cadherin expression in experimental diabetic nephropathy and glucose-stimulated podocytes

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<Title page>

The effect of cyclosporine on P-cadherin expression in experimental diabetic nephropathy and glucose-stimulated podocytes

Directed by Professor Shin-Wook Kang

The Doctoral Dissertation
submitted to the Department of Medicine,
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degree of Doctor of Philosophy

Dong-Ryeol Ryu

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<Signature page>

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배움에 대한 열정과 의욕만으로 대학원 박사 과정에 입학하였습니다. 이 논문이 완성되기까지 수많은 어려움이 있었으나, 옆에서 늘 지켜보아 주시며 힘을 북돋아 주셨던 많은 분들에게 이 글로나마 감사의 마음을 전하고 싶습니다.

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실험 기법에 대한 지식이 전무한 상태에서 하나하나 자상히 알려 주신 허종호 선생님과 강진구 선생님께 감사 드립니다. 그리고 실험을 진행하는 데 있어 많은 도움을 준 친구 유태현 선생님과 실험실의 곽승재 선생님, 정동섭 선생님, 김진주 선생님을 비롯하여, 연세의대 신장내과 교수님들 및 동료 여러분들에게도 고마운 마음을 전합니다.

버팀목처럼 깊은 사랑으로 저를 지켜 주신 부모님들과 가족들, 저에게 무한한 용기를 주는 사랑하는 아내 박윤경, 그리고 한없는 기쁨을 느끼게 하는 아들 기현이에게 감사하며 이 논문을 바칩니다.

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ABSTRACT

The effect of cyclosporine on P-cadherin expression in experimental diabetic nephropathy and glucose-stimulated podocytes

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(Directed by Professor Shin-Wook Kang)

Background: Proteinuria is a clinical characteristic of diabetic nephropathy. The underlying pathologic change responsible for proteinuria is the loss of size-selective and/or charge-selective properties of the glomerular filtration barrier. Size-selectivity is maintained primarily by the slit diaphragm and P-cadherin is known as one of the basic components of it. Cyclosporin A (CsA) has been used in the treatment of various glomerular diseases with proteinuria. However, its mechanism of action has not been fully understood. I investigated whether CsA had the anti-proteinuric effect in diabetic rats and whether it was associated with the alteration of P-cadherin expression. In addition, I evaluated the role of TGF-β_1, which is known to be an upstream activator of calcineurin expression in rat mesangial cells, on P-cadherin expression in cultured podocytes exposed to high glucose medium.

Methods: In vivo, twenty-four Sprague-Dawley rats were injected with diluent (Control, C, N=8) or streptozotocin intraperitoneally. The latter were left untreated (DM, N=8) or treated with 1.5 mg/kg/day CsA (DM+CsA, N=8) for 6 weeks. In vitro, immortalized mouse podocytes were cultured in media

with 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), LG+10 ng/ml TGF-β_1 (LG+TGF-β_1), 30 mM glucose (HG), or HG+10^{-8} M CsA (HG+CsA). RT-PCR and Western blot were performed for P-cadherin and TGF-β_1 mRNA and protein expression, respectively, with sieved glomeruli and cell lysates, and immunofluorescence staining for P-cadherin with renal tissue.

Results:

1. Twenty-four hour urinary albumin excretion at 6 weeks was significantly higher in DM (1.28±0.11 mg/day, $p<0.05$) compared with C rats (0.32 ± 0.02 mg), and CsA treatment inhibited the increase in albuminuria in DM rats (0.62±0.18 mg/day, $p<0.05$).
2. Glomerular calcineurin A-α mRNA and protein expression in DM were 2.1-fold and 2.2-fold higher than C rats, respectively ($p<0.05$). On the other hand, there were no significant differences in calcineurin A-β mRNA and protein expression between the two groups.
3. Glomerular P-cadherin mRNA and protein expression in DM were decreased by 40.2% and 56.1% ($p<0.01$), respectively, compared with C rats, and these decreases were significantly inhibited by CsA ($p<0.05$).
4. Glomerular TGF-β_1 mRNA and protein expression in DM were 2.1-fold and 1.7-fold higher than C rats, respectively ($p<0.05$), and CsA treatment inhibited the increases in TGF-β_1 mRNA and protein expression in DM rats by 56.3% and 76.1%, respectively ($p<0.05$).
5. Immunofluorescence staining for P-cadherin revealed a significant decrease in glomerular P-cadherin expression in DM rats ($p<0.01$), and this decrease was ameliorated by CsA ($p<0.05$).
6. Calcineurin A-α mRNA and protein expression in HG podocytes were 2.2-fold and 1.8-fold higher than LG cells, respectively ($p<0.05$). On the other hand, there were no significant differences in calcineurin A-β mRNA and protein expression between the two groups.

7. P-cadherin mRNA and protein expression in HG podocytes were 50.4% and 66.4% lower than LG cells, respectively ($p<0.05$), and these HG-induced decrements in P-cadherin mRNA and protein expression were restored by CsA ($p<0.05$).
8. LG podocytes treated with TGF-β_1 showed similar changes in P-cadherin mRNA and protein expression as cells cultured under HG medium.
9. TGF-β_1 mRNA and protein expression were 2.4-fold and 1.8-fold higher in HG podocytes compared with LG cells ($p<0.05$), and the increases in TGF-β_1 mRNA and protein expression in HG podocytes were inhibited by 61.2% and 77.9%, respectively, with CsA treatment ($p<0.05$).

Conclusion: I demonstrate for the first time that CsA treatment decreases urinary albumin excretion in 6-weeks DM rats and the decrease in P-cadherin expression in diabetic glomeruli and in HG podocytes is ameliorated by CsA. It suggests that anti-proteinuric effect of CsA may be associated with the alteration of P-cadherin expression in early diabetic nephropathy. In addition, inhibition of the increase in TGF-β_1 expression under diabetic conditions by CsA seems to restore the P-cadherin expression, resulting in the decrease in albuminuria.

Key words: diabetic nephropathy, proteinuria, podocyte, Cyclosporin A, P-cadherin, TGF-β_1

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I. INTRODUCTION

Diabetic nephropathy, the leading cause of end-stage renal disease in the USA[1], is characterized clinically by proteinuria[2]. The underlying pathologic change responsible for proteinuria in various glomerular diseases, including diabetic nephropathy, is the loss of size-selective and/or charge-selective properties of the glomerular filtration barrier[3-7]. The glomerular filtration barrier is comprised of three layers: a fenestrated endothelial layer, glomerular basement membrane (GBM), and podocyte foot processes connected by a slit diaphragm. Traditionally, the GBM has been considered a coarse filter restricting large molecules, while the slit diaphragm was thought to function as a fine filter contributing ultimate size-selectivity, permitting permeability only to molecules smaller than albumin[8].

The slit diaphragm, which bridges adjacent foot processes derived from different podocytes, is a continuous filamentous structure containing pores of 4 x 14 nm, and P-cadherin, nephrin, NEPH1, and FAT are located in the slit

diaphragm region[9-14]. P-cadherin is known to serve as a basic scaffold for the slit diaphragm, and the permselectivity is provided by the slit diaphragm complex composed of P-cadherin and other proteins such as nephrin[10]. Moreover, recent study showed that P-cadherin mRNA and protein expression were decreased in experimental diabetic glomeuli and in podocytes cultured under high glucose medium, suggesting that the changes in glomerular P-cadherin expression may contribute to the development of proteinuria[15].

Diabetic nephropathy is characterized pathologically by glomerular and tubular hypertrophy and increased extracellular matrix (ECM) accumulation[16, 17]. It is well known that elevated blood glucose concentration per se, factors such as transforming growth factor-β_1 (TGF-β_1), angiotensin II, insulin-like growth factor-1 (IGF-1), and signaling pathways such as mitogen-activated protein kinase, lipoxygenase, phosphatidylinositol 3-kinase contribute to these changes[18-22]. Meanwhile, renal hypertrophy and ECM synthesis by IGF-1 and TGF-β_1 required activation of the calcium-dependent serine/threonine phosphatase, calcineurin, and inhibition of calcineurin with Cyclosporin A (CsA) reduced these renal pathologic changes[23, 24].

In the kidney, expression of calcineurin is distributed mainly in proximal tubules, collecting ducts, and in medulla, but also detected in glomeruli[25-28]. Recently, it has been reported that calcineurin expression was upregulated in the diabetic kidney[29]. Moreover, calcineurin is known to be activated by TGF-β and is required for TGF-β-mediated accumulation of ECM proteins in rat mesangial cells[30].

CsA is a known powerful immunosuppressive agent and has been used in the treatment of various glomerular diseases with proteinuria such as minimal change nephrotic syndrome, focal segmental glomerulosclerosis, membranous nephropathy, and lupus nephritis[31-36]. It has been speculated that the anti-proteinuric effect of CsA was related to the hemodynamic change, immunosuppressive effect, direct effect via enhancing size- and/or charge-selectivity of glomerular filtration barrier, or increased levels of glomerular

cAMP[37-39]. Even though some investigators suggested that CsA might alter glomerular permselectivity[37-39], no trial has been made to elucidate the effect of CsA on the expression of glomerular filtration barrier-associated molecules.

In the current study, I investigated whether CsA had anti-proteinuric effect in experimental diabetic rats and whether it was associated with the alteration of P-cadherin expression. In addition, I evaluated the role of TGF-β_1, which is known to be an upstream activator of calcineurin expression in rat mesangial cells, on P-cadherin expression in cultured podocytes exposed to high glucose medium.

II. MATERIALS AND METHODS

1. Animals

All animal studies were conducted under an approved protocol. Twenty-four male Sprague-Dawley rats, weighing 230-270 g were studied. Eight were injected with diluent (Control, C) and 16 were injected with 65 mg/kg streptozotocin (STZ) intraperitoneally. Blood glucose levels were measured on the third day after STZ injection to confirm the development of diabetes. Diabetic rats were then randomly assigned to two groups. CsA doses were carefully determined in view of nephrotoxicity. Signs of chronic nephrotoxicity in rats were observed at doses of CsA >10 mg/kg body weight/day administered for at least 2 weeks[40, 41]. Because this experiment was planned for duration of 6 weeks, I decided a dose of 1.5 mg/kg body weight/day CsA. One group, comprised of 8 rats, was treated with 1.5 mg/kg/day of CsA (Novartis Pharma AG, Basel, Switzerland) (DM+CsA), and the remaining 8 diabetic rats were left untreated (DM). The rats were housed in a temperature-controlled room and were given free access to water and standard laboratory chow. All rats were sacrificed after 6 weeks.

Body weights were checked weekly and kidney weights measured at the time of sacrifice. Blood glucose and 24-hour urinary albumin were also measured at the time of sacrifice. Blood glucose was measured by glucometer and 24-hour urinary albumin excretion was determined by ELISA (Nephrat II, Exocell, Inc., Philadelphia, PA, USA).

2. Podocyte culture

Conditionally immortalized mouse podocytes were kindly provided by Dr. Peter Mundel (Albert Einstein College of Medicine, Bronx, NY, USA) and were cultured as previously described[42]. Briefly, frozen podocytes were first grown under permissive conditions at 33°C in RPMI 1640 media containing

10% fetal bovine serum, 50 U/ml γ-interferon, and 100 U/ml of penicillin/streptomycin in collagen coated flasks, and the γ-interferon tapered down to 10 U/ml in successive passages. Cells were then trypsinized and subcultured without γ-interferon (non-permissive conditions) and allowed to differentiate at 37°C with media changed on alternate days. Differentiation of podocytes at 37°C was confirmed by the identification of synaptopodin, a podocyte differentiation marker, by RT-PCR and Western blotting (data not shown).

After confirming differentiation of podocytes, medium was changed to RPMI medium containing normal glucose (5.6 mM, LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), LG+10 ng/ml TGF-β_1 (Sigma-Aldrich Corp., Saint Louis, MO, USA) (LG+TGF-β_1), high glucose (30 mM, HG), or HG+10^{-8} M CsA (HG+CsA). After 24 hours, cells were harvested for either RNA or protein.

3. RNA isolation and reverse transcription

Glomeruli were isolated by sieving. Purity of the glomerular preparation was greater than 98% as determined by light microscopy. Total RNA was extracted as previously described[20]. Briefly, addition of 100 μl of RNA STAT-60 reagent (Tel-Test, Inc., Friendswood, TX, USA) to the glomeruli was followed by glomerular lysis by freezing and thawing three times. Another 700 μl of RNA STAT-60 reagent was added, the mixture vortexed and stored for 5 minutes at room temperature, 400 μl of chloroform added, and the mixture shaken vigorously for 30 seconds. After 3 minutes, the mixture was centrifuged at 12,000 g for 15 minutes at 4°C and the upper aqueous phase containing the extracted RNA was transferred to a new tube. RNA was precipitated from the aqueous phase by 400 μl isopropanol and pelleted with centrifugation at 12,000 g for 30 minutes at 4°C. The RNA precipitate was washed with 70% ice-cold ethanol, dried using Speed Vac, and dissolved in DEPC-treated distilled water. RNA from podocytes was extracted similarly.

Glomerular and podocytes RNA yield and quality were assessed based on spectrophotometric measures at 260 and 280 nm.

First strand cDNA was made by utilizing a Boehringer Mannheim cDNA synthesis kit (Boehringer Mannheim GmbH, Mannheim, Germany). Two μg of total RNA extracted from sieved glomeruli was reverse transcribed using 10 μM random hexanucleotide primer, 1 mM dNTP, 8 mM $MgCl_2$, 30 mM KCl, 50 mM Tris-HCl, pH 8.5, 0.2 mM dithiothreithol, 25 U RNAse inhibitor, and 40 U avian myeloblastoma virus reverse transcriptase. The mixture was incubated at 30°C for 10 minutes and 42°C for 1 hour, followed by inactivation of enzyme at 99°C for 5 minutes. Podocytes RNA from each plate was similarly reverse transcribed.

4. Real time-polymerase chain reaction (RT-PCR)

The primers and PCR conditions used were shown in Table 1.

Using the ABI PRISM® 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), the PCR was performed in a total volume of 20 μL in each well, containing 10 μL of SYBR Green® PCR Master Mix (Applied Biosystems), 25 ng of cDNA, and 5 pmol sense and antisense primers, which was determined after the analysis of the optimal concentrations of each primer. Each sample was run in triplicates in separate tubes. After PCR, the temperature was increased from 60 to 95°C to construct a melting curve. A control without cDNA was run in parallel with each assay.

The cDNA content of each specimen was determined using comparative C_T method with $2^{-\Delta\Delta CT}$. The results were given as relative expression of P-cadherin, calcineurin A-α, calcineurin A-β, and TGF-β_1 normalized to the GAPDH housekeeping gene. Signals from control glomeruli or LG podocytes were considered a relative value of 1.0. In pilot experiments, PCR products run on 2% agarose gels using each primer revealed a single band (Figure 1).

Table 1. Sequences of primers and PCR conditions

	Sequence (5'→3')	Annealing Temperature (°C)
Rat and mouse TGF-β_1		
Sense	AAACGGAAGCGCATCGAA	62
Antisense	GGGACTGGCGAGCCTTAGTT	
Rat P-cadherin		
Sense	AGTGGGCCACGAGGTACAGA	53
Antisense	ACGCCATGCCGGTGAGT	
Mouse P-cadherin		
Sense	TGTATCTGAGAATGGTGCCTCTGTAG	56
Antisense	TTGAGTGAACTTGGGCTTGTTG	
Rat GAPDH		
Sense	TGCCAAGTATGATGACATCAAGAAG	60
Antisense	AGCCCAGGATGCCCTTTAGT	
Mouse GAPDH		
Sense	TGTGTCCGTCGTGGATCTGA	60
Antisense	CCTGCTTCACCACCTTCTTGA	
Rat and Mouse calcineurin A-α		
Sense	CGAGCCCAAGGCGATTG	60
Antisense	GGAAATGGAACGGCTTTCAC	
Rat and Mouse calcineurin A-β		
Sense	TTCCCTGAACACCGCACAT	60
Antisense	CTGGTCACTGGGCACTATGGT	

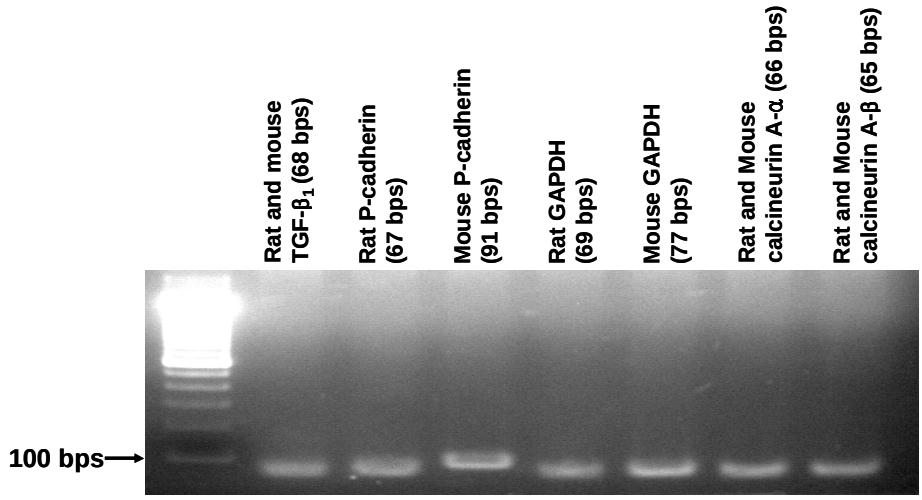


Figure 1. PCR products run on 2% agarose gel electrophoresis using each primer revealed a single band.

5. Western blot analysis

Sieved glomeruli and podocytes harvested from plates were lysed in sodium dodecyl sulfate (SDS) sample buffer (2% sodium dodecyl sulfate, 10 mM Tris-HCl, pH 6.8, 10% [vol/vol] glycerol). Lysate was centrifuged at 10,000 g for 10 minutes at 4°C and the supernatant was stored at -70°C until all rats were sacrificed. Protein concentrations were determined with a Bio-Rad kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Aliquots of 50 μg protein were treated with Laemmli sample buffer, then heated at 100°C for 5 minutes, and electrophoresed 50 μg/lane in 8% or 12% acrylamide denaturing SDS-polyacrylamide gel. Proteins were transferred to Hybond-ECL membrane (Amersham Life Science, Inc., Arlington Heights, IL, USA) using a Hoeffer semidry blotting apparatus (Hoeffer Instruments, San Francisco, CA, USA), the membrane incubated in blocking buffer A (1 X PBS, 0.1% Tween-20, and 8% non-fat milk) for 1 hour at room temperature, and then incubated overnight at 4°C with a 1:500 dilution of monoclonal anti-P-cadherin antibody (Zymed Laboratories, Inc., San Francisco, CA, USA), polyclonal anti-TGF-β_1 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), polyclonal anti-calcineurin A-α or A-β antibody (Chemicon International, Inc., Temecula, CA, USA), or polyclonal anti-β-actin antibody (Santa Cruz Biotechnology, Inc.). The membrane was washed once for 15 minutes and twice for 5 minutes in 1 X PBS with 0.1% Tween-20, and incubated in buffer A with horseradish peroxidase-linked sheep anti-mouse IgG (Amersham Life Science, Inc.) at 1:1000 dilution. The washes were repeated, and the membrane developed with chemiluminescent agent (ECL; Amersham Life Science, Inc.).

6. Glomerular pathology

Slices of kidney for immunofluorescence staining were fixed in 10% neutral buffered formalin, processed in the standard manner, and 5 micron

sections of paraffin embedded tissues were utilized. Slides were deparaffinized, hydrated in ethyl alcohol, and washed in tap water. Antigen retrieval was carried out in 10 mM sodium citrate buffer for 20 minutes using a Black and Decker vegetable steamer. For P-cadherin staining, monoclonal anti-P-cadherin antibody (Zymed Laboratories, Inc.) was diluted in 1:200 with 2% casein in BSA and was applied for overnight incubation at room temperature. After washing, a secondary Rhodamine Red-X-conjugated goat anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was added for 60 minutes. A semiquantitative score for measuring P-cadherin immunofluorescence intensity within glomeruli was determined by examining thirty glomeruli in each section and by digital image analysis (MetaMorph version 4.6r5, Universal Imaging Corp., Downingtown, PA) as previously described[43]. To confirm that the change of P-cadherin expression was not due to the change of podocyte numbers, immunohistochemical staining for Wilms' tumor-1 protein (WT-1) was also done. A rabbit polyclonal antibody to WT-1 (Santa Cruz Biotechnology, Inc.) was diluted in 1:100 with 2% casein in BSA and was applied for overnight incubation at room temperature. After washing, a secondary goat anti-rabbit antibody was added for 20 minutes, the slides were washed and incubated with a tertiary rabbit-PAP complex for 20 min. DAB was added for 2 minutes and the slides were counterstained with hematoxylin. All cells stained positive for WT-1 in 20 glomeruli cut at the vascular pole were considered as podocytes and were counted to confirm the change of podocyte numbers.

7. Statistical analyses

Results are expressed as the mean ± SEM. Statistical analyses were performed using the statistical package SPSS for Windows Version 11.0 (SPSS, Inc., Chicago, IL, USA). Results were analyzed using Kruskal-Wallis non-parametric test for multiple comparisons. If there was a significant difference by the Kruskal-Wallis test, it was further confirmed by the Mann-

Whitney U test. Statistical significance was determined, when P values were less than 0.05.

III. RESULTS

1. Animal studies

A. Animal data

Body weight increased in all the three groups, but increased more in C (402±6 g) than in DM (270±4 g) and DM+CsA rats (299±8 g) ($p<0.05$). Kidney weight was measured at the time of sacrifice. The ratio of kidney weight to body weight in DM (1.09±0.08%) and DM+CsA (1.01±0.08%) rats was significantly higher than C rats (0.76±0.05%) ($p<0.05$).

The mean blood glucose levels of C, DM, and DM+CsA rats were 95.8±0.9 mg/dl, 466.2±13.9 mg/dl, and 495.1±13.8 mg/dl, respectively ($p<0.01$). Compared to the C group (0.32±0.02 mg/day), 24-hour urinary albumin excretion at 6 weeks was significantly higher in DM rats (1.28±0.11 mg/day, $p<0.05$) and CsA treatment inhibited the increase in albuminuria in DM rats (0.62±0.18 mg/day, $p<0.05$) (Table 2).

Table 2. Animal data of the three groups

	Control (N=8)	DM (N=8)	DM+CsA (N=8)
Body weight after 6 weeks (g)	402 ± 6	270 ± 4*	299 ± 8*
Kidney Wt/Body Wt (%)	0.76 ± 0.05	1.09 ± 0.08*	1.01 ± 0.08*
Blood glucose (mg/dl)	95.8 ± 0.9	466.2 ± 13.9$^{\$}$	495.1 ± 13.8$^{\$}$
Urinary albumin excretion (mg/day)	0.32 ± 0.02	1.28 ± 0.11*	0.62 ± 0.18$^{\#}$

*, $p<0.05$ vs. Control; $^{\#}$, $p<0.05$ vs. DM; $^{\$}$, $p<0.01$ vs. Control.

B. Glomerular calcineurin A-α and A-β mRNA and protein expression

Calcineurin A-α mRNA expression was 2.1-fold higher in DM compared with C glomeruli ($p<0.05$). On the other hand, there were no significant differences in calcineurin A-β and GAPDH mRNA expression between the two groups (Figure 2).

Western blotting experiments revealed that glomerular calcineurin A-α protein expression was increased in DM by 123% compared with C rats ($p<0.05$), while there were no significant differences in calcineurin A-β and β-actin protein expression between the two groups (Figure 3).

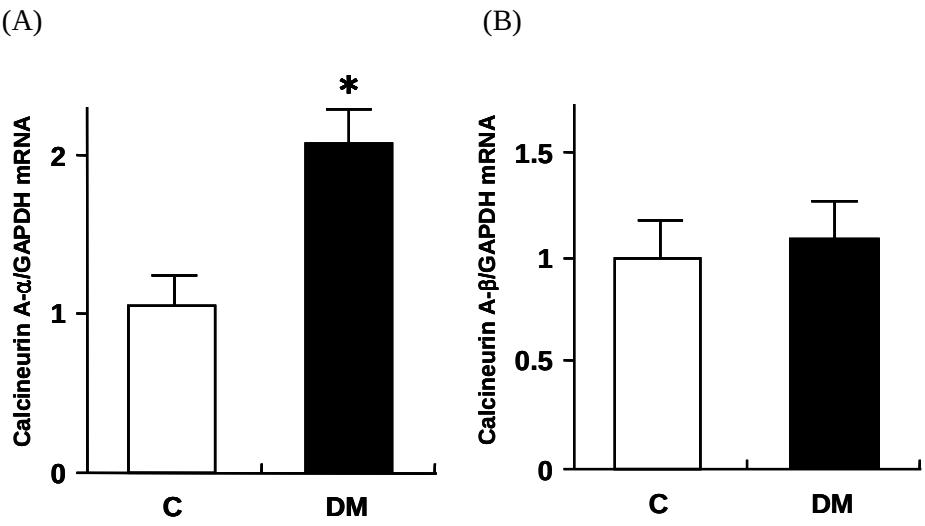
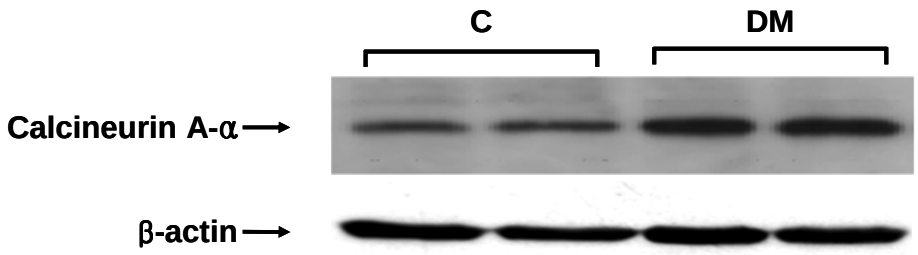


Figure 2. Calcineurin A-α/GAPDH mRNA and calcineurin A-β/GAPDH mRNA ratio in control (C) and DM glomeruli at 6 weeks after induction of DM. (A) Calcineurin A-α/GAPDH mRNA ratio was significantly higher in DM than C glomeruli. (B) On the other hand, there was no significant difference in calcineurin A-β/GAPDH mRNA ratio between the two groups.
*, $p<0.05$ vs. C.

(A)



(B)

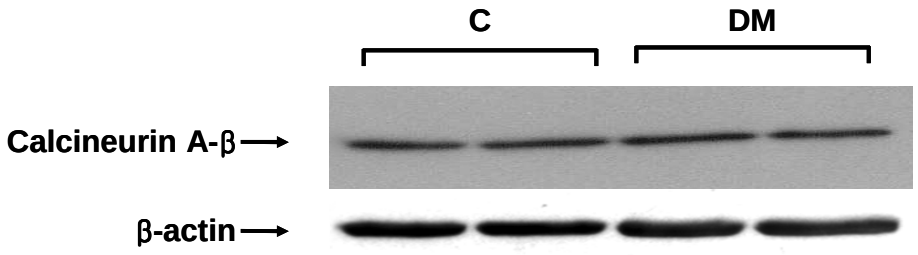


Figure 3. A representative Western blot for calcineurin A-α and A-β with glomerular lysates of control (C) and DM rats at 6 weeks after induction of DM (representative of five blots). (A) Calcineurin A-α protein expression was significantly increased in DM compared with C glomeruli ($p<0.05$). (B) There were no differences in calcineurin A-β and β-actin protein expression between the two groups.

C. Glomerular P-cadherin and TGF-β_1 mRNA and protein expression

Glomerular P-cadherin mRNA expression in DM was decreased by 40.2% compared with C rats at 6 weeks after induction of DM ($p<0.01$), and this decrease was significantly inhibited with CsA treatment (Figure 4). In addition, glomerular TGF-β_1 mRNA expression in DM was 2.1-fold higher than C rats, and CsA treatment inhibited the increase in TGF-β_1 mRNA expression in DM rats by 56.3% ($p<0.05$) (Figure 5). In contrast, there was no significant difference in the amount of glomerular GAPDH mRNA among the three groups.

Figure 6 shows a representative Western blot for P-cadherin of equal amounts of protein from the lysates of sieved C, DM, and DM+CsA glomeruli at 6 weeks. Glomerular P-cadherin protein expression was also decreased in DM compared with C and was restored in DM+CsA rats as P-cadherin mRNA expression. Densitometric quantitation revealed that there was a 56.1% decrease in P-cadherin protein expression in DM relative to C glomeruli ($p<0.01$), and this decrease was significantly inhibited with CsA treatment. As shown in Figure 7, TGF-β_1 protein levels were 1.7-fold higher in DM compared with C glomeuli, and CsA treatment inhibited the increase in glomerular TGF-β_1 protein expression in DM rats by 76.1% ($p <0.05$). In contrast, there was no difference in β-actin protein expression among the three groups.

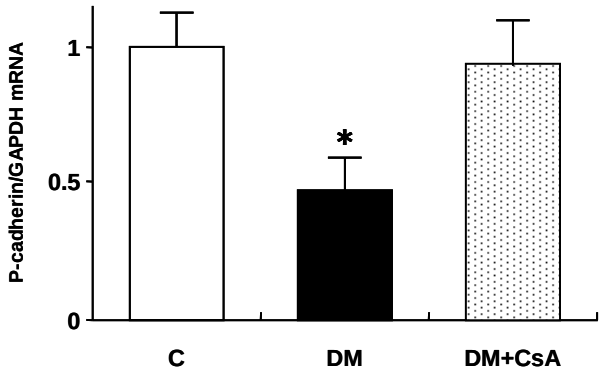


Figure 4. Glomerular P-cadherin/GAPDH mRNA in control (C), DM, and DM rats treated with 1.5 mg/kg/day CsA (DM+CsA) for 6 weeks. Glomerular P-cadherin/GAPDH mRNA ratio was significantly lower in DM compared with C rats at 6 weeks after induction of DM, and this decrease was significantly inhibited with CsA treatment.

*, $p<0.01$ vs. C and DM+CsA.

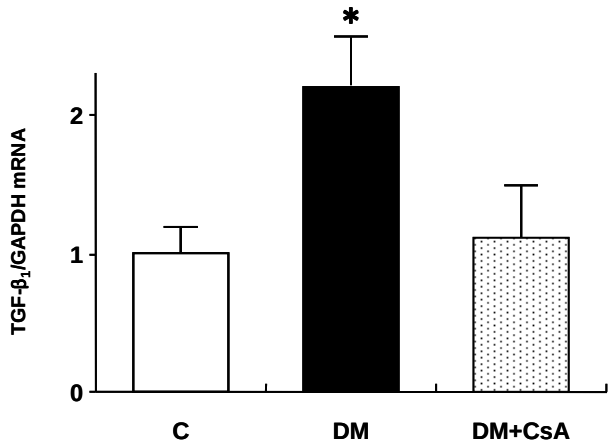


Figure 5. Glomerular TGF-β_1/GAPDH mRNA in control (C), DM, and DM rats treated with 1.5 mg/kg/day CsA (DM+CsA) for 6 weeks. Glomerular TGF-β_1/GAPDH mRNA ratio in DM was significantly higher compared with C rats at 6 weeks after DM induction, and CsA treatment significantly inhibited the increase in glomerular TGF-β_1/GAPDH mRNA ratio in DM rats.

*, $p<0.05$ vs. C and DM+CsA.

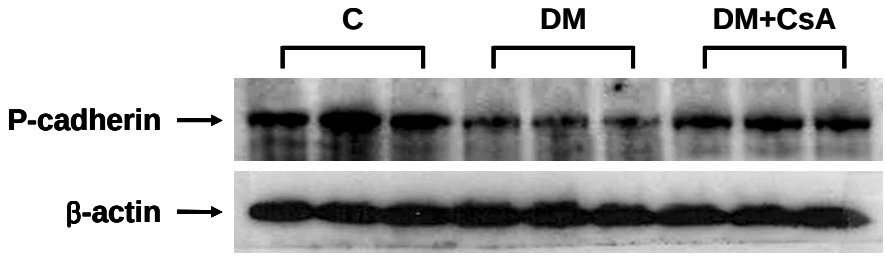


Figure 6. A representative Western blot for P-cadherin with glomerular lysates of control (C), DM, and DM rats treated with 1.5 mg/kg/day CsA (DM+CsA) for 6 weeks (representative of five blots). Densitometric quantitation revealed that there was a 56.1% decrease in glomerular P-cadherin protein expression in DM relative to C rats ($p<0.01$), and this decrease was significantly inhibited with CsA treatment ($p<0.05$). In contrast, there was no difference in β-actin protein expression among the three groups.

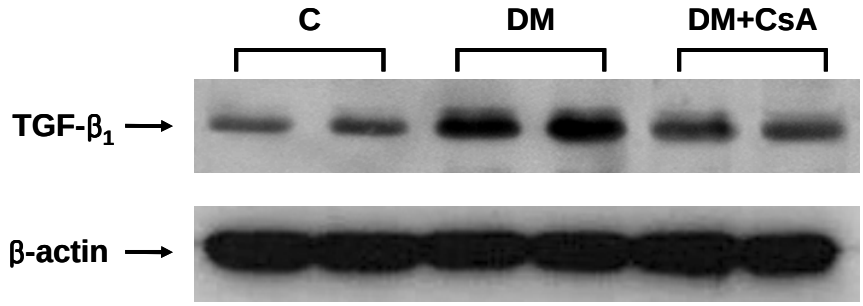
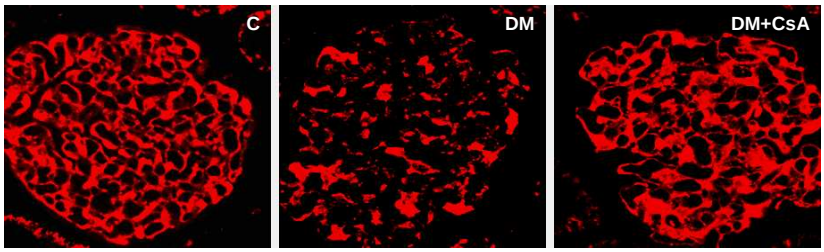


Figure 7. A representative Western blot for TGF-β_1 with glomerular lysates of control (C), DM, and DM rats treated with 1.5 mg/kg/day CsA (DM+CsA) for 6 weeks (representative of five blots). TGF-β_1 protein levels were 1.7-fold higher in DM compared with C glomeruli, and CsA treatment inhibited the increase in glomerular TGF-β_1 protein expression in DM rats by 76.1% ($p<0.05$). In contrast, there was no difference in β-actin protein expression among the three groups.

D. Pathologic findings

Immunofluorescence staining for glomerular P-cadherin confirmed the mRNA and Western blot findings. There was a significant decrease in glomerular P-cadherin expression, which exhibited linear/punctate distribution along the glomerular capillary loops, in DM rats. The decrease in P-cadherin expression in DM glomeruli was ameliorated by CsA treatment (Figure 8A). Semiquantitative score of immunofluorescence intensity for P-cadherin was significantly lower in DM compared with C ($p<0.01$) and this decrease was significantly ameliorated by CsA ($p<0.05$) (Figure 8B). In contrast, there was no statistical difference in mean podocyte counts per glomerulus assessed by WT-1 staining among the three groups (C, 15.8±1.8; DM, 15.3±1.9; DM+CsA, 16.1±2.0).

(A)



(B)

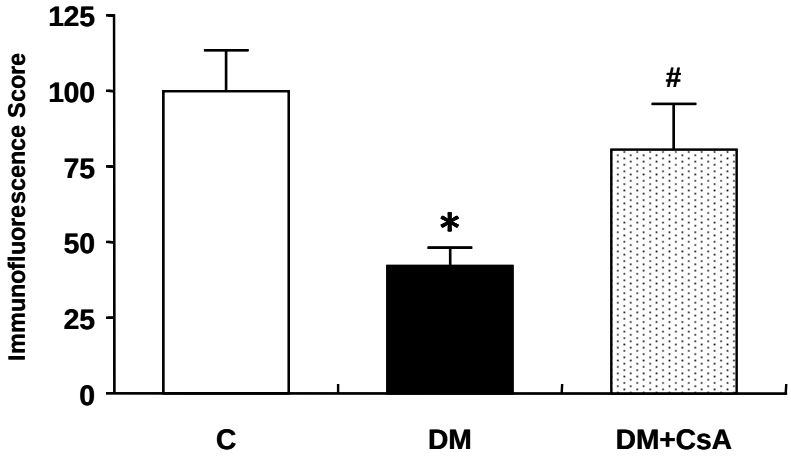


Figure 8. Immunofluorescence staining for P-cadherin protein in control (C), DM, and DM rats treated with 1.5 mg/kg/day CsA (DM+CsA) for 6 weeks. (A) There was a significant decrease in glomerular P-cadherin expression, which exhibited linear/punctate distribution along the glomerular capillary loops, in DM rats. The decrease in P-cadherin expression in DM glomeruli was ameliorated by CsA. (x 400) (B) Semiquantitative immunofluorescence score for glomerular P-cadherin was significantly lower in DM compared with C and DM+CsA rats.

*, $p<0.01$ vs. C; $^{\#}$, $p<0.05$ vs. DM.

2. Podocyte culture studies

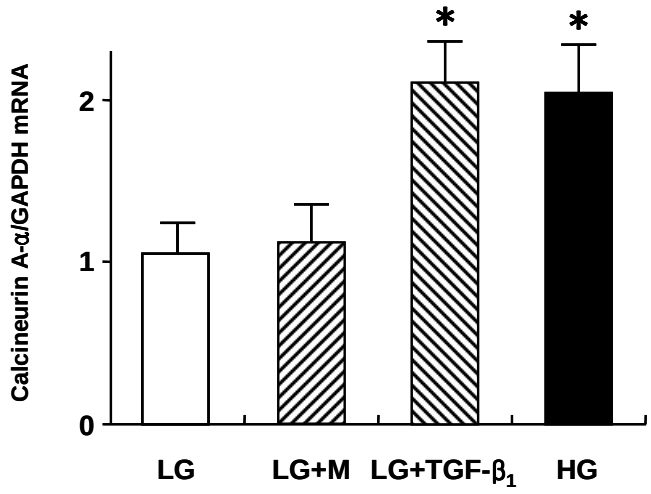
A. Calcineurin A-α and A-β mRNA and protein expression

Calcineurin A-α mRNA expression was 2.2-fold higher in podocytes exposed to HG than those exposed to LG. Similarly, there was also a 2.3-fold increment of calcineurin A-α mRNA expression in podocytes exposed to LG+TGF-β_1 compared with LG cells ($p<0.05$) (Figure 9A). On the other hand, there were no significant differences in calcineurin A-β and GAPDH mRNA expression (Figure 9B).

Western blotting experiments revealed similar findings as RT-PCR. Calcineurin A-α protein expression was increased in podocytes exposed to HG and LG+TGF-β_1 by 83% and 67%, respectively, compared with LG cells ($p<0.05$), while there were no significant differences in calcineurin A-β and β-actin protein expression (Figure 10).

Mannitol (24.4 mM) had no effect on calcineurin A-α and A-β mRNA and protein expression in podocytes.

(A)



(B)

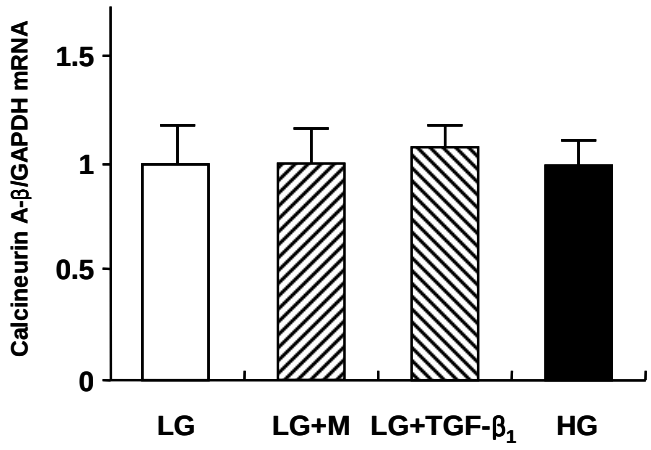
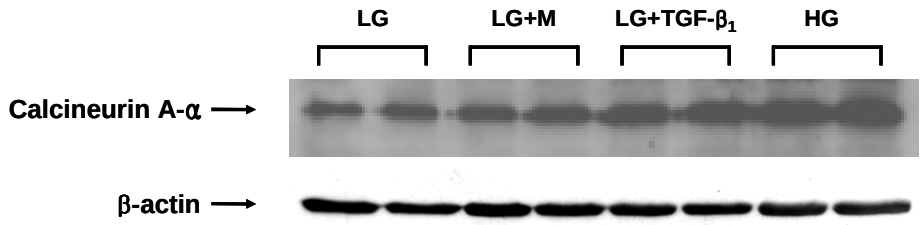


Figure 9. Calcineurin A-α/GAPDH mRNA and calcineurin A-β/GAPDH mRNA in podocytes exposed to 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10 ng/ml TGF-β_1 (LG+TGF-β_1), or 30 mM glucose (HG) medium. (A) Calcineurin A-α/GAPDH mRNA ratio was significantly higher in podocytes exposed to LG+TGF-β_1 and HG than those exposed to LG. (B) There was no significant difference in calcineurin A-β/GAPDH mRNA ratio.

*, $p<0.05$ vs. C.

(A)



(B)

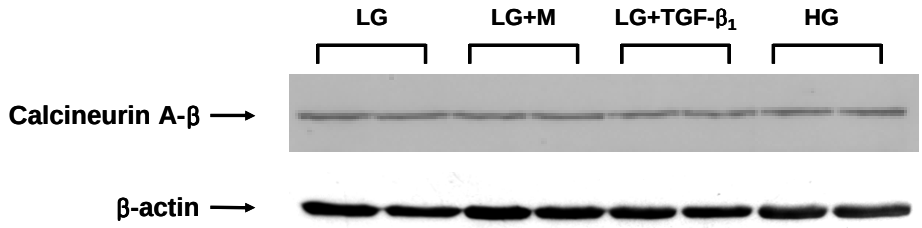


Figure 10. A representative Western blot for calcineurin A-α and A-β with podocytes exposed to 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10 ng/ml TGF-β_1 (LG+TGF-β_1), or 30 mM glucose (HG) medium (representative of four blots). (A) Calcineurin A-α protein expression was significantly increased in podocytes exposed to LG+TGF-β_1 and HG compared with LG cells ($p<0.05$). (B) There were no differences in calcineurin A-β and β-actin protein expression.

B. P-cadherin and TGF-β_1 mRNA and protein expression

P-cadherin mRNA expression of podocytes exposed to HG was 50.4% lower than that of LG cells, and this HG-induced decrement in P-cadherin mRNA expression was ameliorated by CsA ($p<0.05$). In addition, P-cadherin mRNA expression was also decreased by 49.3% in podocytes exposed to LG+TGF-β_1 compared with LG cells ($p<0.05$) (Figure 11). In contrast, TGF-β_1 mRNA expression was 2.4-fold higher in podocytes exposed to HG compared with LG cells, and this increase in TGF-β_1 mRNA expression in HG podocytes was inhibited by 61.2% with CsA treatment ($p<0.05$) (Figure 12). There was no significant difference in GAPDH mRNA expression.

P-cadherin protein expression was also significantly lower in podocytes exposed to HG and LG+TGF-β_1 than LG cells. There were 66.4% and 67.5% decrease in P-cadherin protein expression in podocytes exposed to HG and LG+TGF-β_1, respectively, compared with LG cells assessed by densitometry ($p<0.05$), and CsA nearly normalized this HG-induced reduction in P-cadherin protein expression (Figure 13). Figure 14 shows a representative Western blot for TGF-β_1 protein expression in podocytes exposed to LG, LG+M, LG+CsA, HG, and HG+CsA. There was a 1.8-fold increase in TGF-β_1 protein expression in HG podocytes compared with LG cells ($p<0.05$), and CsA inhibited the increase in TGF-β_1 protein expression in HG cells by 77.9% ($p<0.05$).

Mannitol (24.4 mM) and CsA (10^{-8} M) had no effect on P-cadherin and TGF-β_1 mRNA and protein expression in LG podocytes.

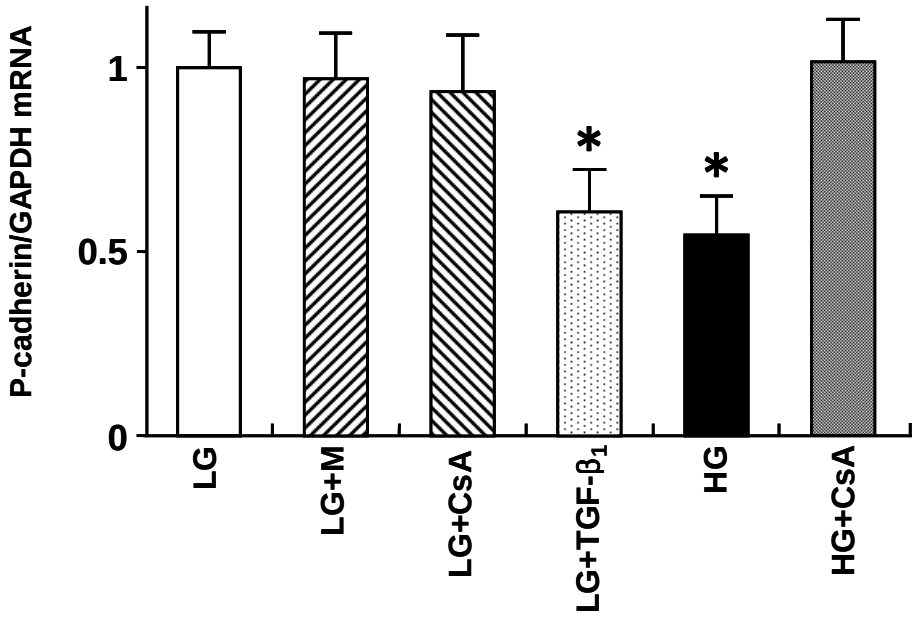


Figure 11. P-cadherin/GAPDH mRNA in podocytes exposed to 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), LG+10 ng/ml TGF-β_1 (LG+TGF-β_1), 30 mM glucose (HG), or HG+10^{-8} M CsA (HG+CsA) medium. P-cadherin/GAPDH mRNA ratio of podocytes exposed to HG and LG+TGF-β_1 was significantly lower than that of LG cells, and this HG-induced decrement in P-cadherin/GAPDH mRNA ratio was ameliorated by CsA.

*, $p<0.05$ vs. LG and HG+CsA.

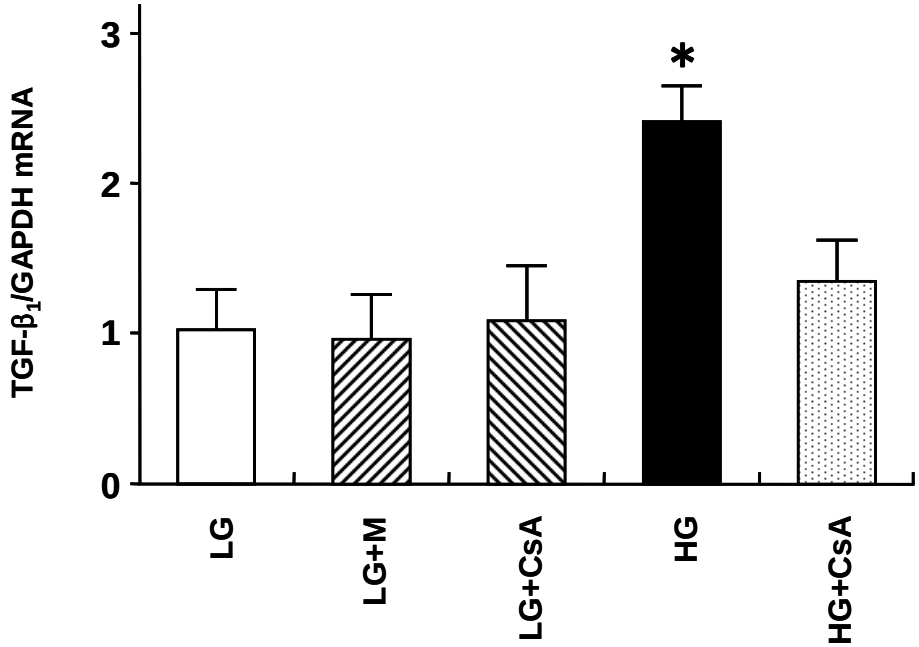


Figure 12. TGF-β_1/GAPDH mRNA in podocytes exposed to 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), 30 mM glucose (HG), or HG+10^{-8} M CsA (HG+CsA) medium. TGF-β_1/GAPDH mRNA ratio was significantly higher in podocytes exposed to HG compared with LG cells, and this increase in TGF-β_1/GAPDH mRNA ratio in HG podocytes was inhibited with CsA treatment.

*, $p<0.05$ vs. LG and HG+CsA.

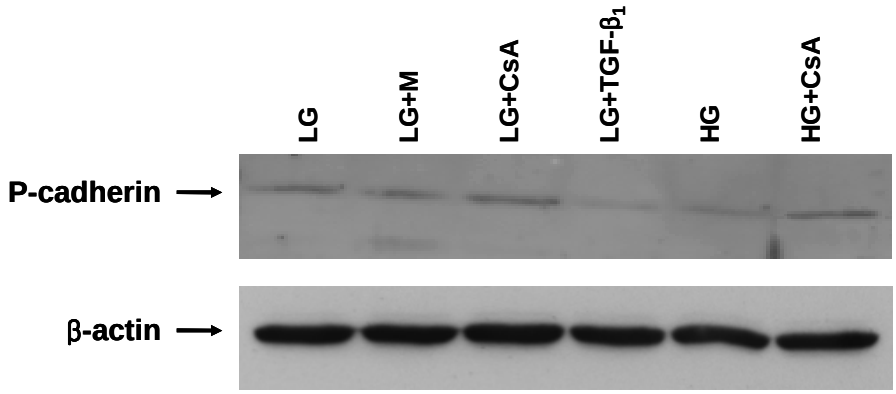


Figure 13. A representative Western blot for P-cadherin in podocytes exposed to 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), LG+10 ng/ml TGF-β_1 (LG+TGF-β_1), 30 mM glucose (HG), or HG+10^{-8} M CsA (HG+CsA) (representative of five blots). P-cadherin protein expression was significantly lower in HG and LG+TGF-β_1 podocytes than LG cells assessed by densitometry ($p<0.05$), and CsA nearly normalized this HG-induced reduction in P-cadherin protein expression ($p<0.05$).

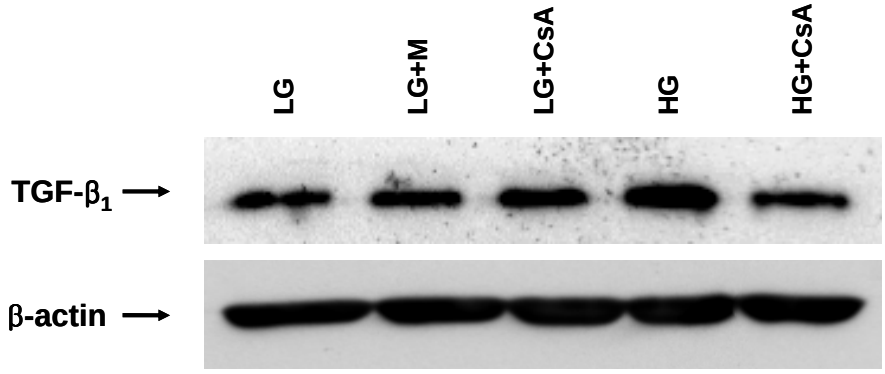


Figure 14. A representative Western blot for TGF-β_1 in podocytes exposed to 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), 30 mM glucose (HG), or HG+10^{-8} M CsA (HG+CsA) (representative of five blots). TGF-β_1 protein expression was significantly increased in HG podocytes compared with LG cells ($p<0.05$), and CsA inhibited the increase in TGF-β_1 protein expression in HG cells ($p<0.05$).

IV. DISCUSSION

In this study, I demonstrate for the first time that CsA treatment for 6 weeks reduced albuminuria in experimental diabetic rats. This study also shows that the anti-proteinuric effect of CsA seems to result from inhibition of the increase in TGF-β_1 expression and restoration of the decrease in P-cadherin expression, both in vivo and in vitro.

CsA is a well known powerful immunosuppressant consisting of 11 amino acids. The mechanisms of action of CsA involve reduction of interleukin-2 and cytokine production by inhibiting calcineurin activation[44]. Calcineurin is a serine/threonine phosphatase and is considered as a messenger in the dephosphrylation and activation of the nuclear factor of activated T cell family, myocyte enhancer-binding factors, and GATA proteins[45]. It is comprised of the catalytic subunit A and regulatory subunit B, and the A subunit has three isoforms: α, β, and γ. The α and β isoforms are widely expressed in most organs, whereas the γ isoform is expressed predominantly in the testes[46]. In the kidney, calcineurin activity was relatively high in proximal and distal tubules which corresponded to the expression of predominantly the α isoform[27, 28]. Gooch et al[29] revealed that all calcineurin A isoforms were upregulated in STZ-induced diabetic rat kidney, but increment of only calcineurin A-α expression was observed in glomeruli. Using double immunofluorescent labeling, they demonstrated that the cells with increased expression of calcineurin A-α isoform were endothelial and mesangial cells. In this study, I provide evidence that calcineurin A-α was also upregulated in diabetic glomeruli and cultured podocytes exposed to high glucose, suggesting that activated calcineurin may play a role in gene regulation in podocytes under diabetic conditions.

In addition to immunosuppressive therapy, CsA has been used for proteinuria in various glomerular diseases, including minimal change disease, focal segmental glomerulosclerosis, and membranous nephropathy[31-35]. The mechanisms of action in the treatment of these diseases have been speculated

as follows: 1) inhibition of production of cytokines which is thought to be of T-cell origin and is supposed to be important in the pathogenesis of certain glomerular diseases; 2) hemodynamic effects; 3) effects on charge-selective properties of the GBM; and 4) effects on size-selective properties of glomerular filtration barrier[37-39]. Even though some investigators suggested that CsA might alter glomerular permselectivity[37-39], no trial has been made to elucidate the effect of CsA on the expression of glomerular filtration barrier-associated molecules. In this study, I demonstrate for the first time that P-cadherin, a molecule known to be located at the slit diaphragm, expression was altered by CsA.

P-cadherin is a 120,000 M_r transmembrane protein that belongs to a large cell-cell adhesion superfamily with calcium-dependent homophilic adhesion properties. It consists of five cadherin domains at its extracellular portion and a β-catenin binding site at its cytoplasmic part, through which the molecule is linked to the actin cytoskeleton[47]. As homophilic interactions was demonstrated for cadherins at the molecular level[48], it has been supposed that P-cadherin constitutes the core protein of the slit diaphragm, whereas the permselectivity is provided by the slit diaphragm complex composed of P-cadherin and other proteins such as nephrin[10]. Nevertheless, the role of P-cadherin in the pathogenesis of proteinuria has been less studied. In congenital Finnish type nephrotic kidney, P-cadherin was expressed normally in spite of absent slit diaphragms[49]. In addition, even though proteinuria was not investigated in P-cadherin-deficient mice, it seemed that life-threatening proteinuria did not develop in view of the survival of these mice[50]. In contrast, Liu et al[51] demonstrated that intravenous injection of anti-P-cadherin antibody resulted in 49% increase in 24-hour urinary protein excretion independent of nephrin or NEPH1, suggesting that P-cadherin also serve as a glomerular filtration barrier to protein. A more recent study also revealed that P-cadherin mRNA and protein expression were decreased in diabetic glomeruli and cultured podocytes exposed to high glucose, suggesting the potential role of

P-cadherin in the development of proteinuria in diabetic nephropathy[15].

Despite the beneficial effects of CsA on organ transplantation and various steroid-resistant nephrotic syndrome, the clinical use of CsA is often limited by nephrotoxicity. Chronic CsA nephrotoxicity is characterized pathologically by afferent arteriolopathy, tubulointerstitial fibrosis, and interstitial inflammatory cell infiltration[52, 53]. The mechanism responsible for this complication is not well understood, but low grade ischemia and various mediators have been implicated[54, 55]. One of the important mediators is TGF-β_1. Both in vivo and in vitro studies have demonstrated that TGF-β_1 expression is increased by CsA. CsA induced TGF-β_1 mRNA and protein expression in activated human T cells, human lung adenocarcinoma cells, and mink lung epithelial cell line[56, 57]. In addition, Wolf et al[58] also observed that TGF-β_1 protein expression in proximal tubular cells and tubulointerstitial fibroblasts was increased by CsA in a dose-dependent manner. Furthermore, CsA treatment for 2 weeks increased renal cortical TGF-β_1 mRNA expression and administration of specific TGF-β neutralizing antibody ameliorated tubulointerstitial changes in a mouse model of chronic CsA nephrotoxicity[59]. In contrast, the effect of CsA on TGF-β_1 mRNA expression was completely different between glomeruli and renal cortex. Gooch et al[24] observed decreased rather than increased TGF-β_1 mRNA expression in diabetic glomeruli by CsA, which was in concord with the results of this study. The divergence in the effect of CsA on TGF-β_1 expression is not clear, but differences in experimental animals, cell types, duration of CsA treatment, or doses of CsA administered may contribute.

In the rat, chronic CsA nephrotoxicity develops at doses greater than 10 mg/kg body weight administered for at least 2 weeks[40, 41]. A recent study also demonstrated that there was no evidence of CsA nephrotoxicity in rats given 5 mg/kg body weight CsA for 2 weeks[24]. Since the purpose of this study was to clarify the effect of CsA on urinary albumin excretion, CsA treatment was scheduled for 6 weeks when urinary albumin excretion became evident in

diabetic rats. Therefore, the dose of 1.5 mg/kg body weight was determined to make the total amount of CsA administered similar to the previous study[24]. As a result, no signs of CsA nephrotoxicity were observed histologically in CsA-treated rats of this study.

CsA is a diabetogenic drug in clinical field especially after organ transplantation[60]. In contrast, blood glucose levels were decreased in diabetic rats by low dose CsA treatment for 14 days, however, the reason for this hypoglycemic effect was not clearly described[24]. In this study, there was no difference in blood glucose levels between diabetic and CsA-treated diabetic rats. Therefore, the anti-proteinuric effect of CsA in these diabetic rats was not associated with the changes of blood glucose levels.

In conclusion, urinary albumin excretion was reduced in CsA-treated diabetic rats and changes in P-cadherin and TGF-β_1 expression under diabetic conditions, both in vivo and in vitro, were inhibited by CsA. Taken together, the anti-proteinuric effect of CsA seems to be closely related to the inhibition of TGF-β_1 expression, resulting in the restoration of decreased P-cadherin expression under diabetic conditions.

V. CONCLUSION

I investigated whether CsA had anti-proteinuric effect in diabetic rats and whether it was associated with the alteration of P-cadherin expression. In addition, I evaluated the role of TGF-β_1 on P-cadherin expression in cultured podocytes exposed to high glucose medium.

In vivo, twenty-four Sprague-Dawley rats were divided into three groups, control (C), DM, and DM+CsA. In vitro, immortalized mouse podocytes were cultured in media with 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), LG+10^{-8} M CsA (LG+CsA), LG+10 ng/ml TGF-β_1 (LG+TGF-β_1), 30 mM glucose (HG), or HG+10^{-8} M CsA (HG+CsA). RT-PCR and Western blot were performed for P-cadherin and TGF-β_1 mRNA and protein expression, respectively, with sieved glomeruli and cell lysates, and immunofluorescence staining for P-cadherin with renal tissue.

1. Twenty-four hour urinary albumin excretion at 6 weeks was significantly higher in DM (1.28±0.11 mg/day, $p<0.05$) compared with C rats (0.32 ± 0.02 mg), and CsA treatment inhibited the increase in albuminuria in DM rats (0.62±0.18 mg/day, $p<0.05$).
2. Glomerular calcineurin A-α mRNA and protein expression in DM were 2.1-fold and 2.2-fold higher than C rats, respectively ($p<0.05$). On the other hand, there were no significant differences in calcineurin A-β mRNA and protein expression between the two groups.
3. Glomerular P-cadherin mRNA and protein expression in DM were decreased by 40.2% and 56.1%, respectively ($p<0.01$), compared with C rats, and these decreases were significantly inhibited by CsA ($p<0.05$).
4. Glomerular TGF-β_1 mRNA and protein expression in DM were 2.1-fold and 1.7-fold higher than C rats, respectively ($p<0.05$), and CsA treatment inhibited the increases in TGF-β_1 mRNA and protein expression in DM rats by 56.3% and 76.1%, respectively ($p<0.05$).

5. Immunofluorescence staining for P-cadherin revealed a significant decrease in glomerular P-cadherin expression in DM rats ($p<0.01$), and this decrease in P-cadherin expression was ameliorated by CsA ($p<0.05$).
6. Calcineurin A-α mRNA and protein expression in HG podocytes were 2.2-fold and 1.8-fold higher than LG cells, respectively ($p<0.05$). On the other hand, there were no significant differences in calcineurin A-β mRNA and protein expression between the two groups.
7. P-cadherin mRNA and protein expression in HG podocytes were 50.4% and 66.4% lower than LG cells, respectively ($p<0.05$), and these HG-induced decrements in P-cadherin mRNA and protein expression were restored by CsA ($p<0.05$).
8. LG podocytes treated with TGF-β_1 showed similar changes in P-cadherin mRNA and protein expression as cells cultured under HG medium.
9. TGF-β_1 mRNA and protein expression were 2.4-fold and 1.8-fold higher in HG podocytes compared with LG cells ($p<0.05$), and the increases in TGF-β_1 mRNA and protein expression in HG podocytes were inhibited by 61.2% and 77.9%, respectively, with CsA treatment ($p<0.05$).

In conclusion, I demonstrate for the first time that CsA treatment decreases urinary albumin excretion in 6-weeks DM rats and the decrease in P-cadherin expression under diabetic conditions, both in vivo and in vitro, is ameliorated by CsA. It suggests that anti-proteinuric effect of CsA may be associated with the alteration of P-cadherin expression in early diabetic nephropathy. In addition, inhibition of increased TGF-β_1 expression under diabetic conditions by CsA seems to restore the P-cadherin expression, resulting in the decrease in albuminuria.

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ABSTRACT (in Korean)

당뇨 백서 사구체 및 고포도당으로 자극한 족세포에서 싸이클로스포린이 P-cadherin의 발현에 미치는 영향

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배 경: 당뇨병성 신병증은 말기 신부전증의 원인 질환 중 가장 많은 빈도를 차지하는 질환으로, 특징적인 임상 소견인 단백뇨는 사구체 여과 장벽 (glomerular filtration barrier)의 크기 선택성 (size selectivity) 또는 전하 선택성 (charge selectivity)의 소실로 인해 발생되는 것으로 알려져 있다. 세극막은 사구체 여과 장벽의 구성 성분인 족세포에서 기원한 족돌기들을 연결하는 구조로, 여기에는 P-cadherin 등의 단백이 존재하는 것으로 보고되어 있다. 싸이클로스포린은 장기 이식 후 흔히 사용하는 면역억제제일 뿐만 아니라, 여러 사구체 질환에서 단백뇨 감소 효과가 있는 것으로 알려져 있으나, 당뇨병성 신병증에서의 효과에 대한 연구는 미미한 실정이다. 따라서 본 연구에서는 여러 사구체 질환에서 관찰되는 싸이클로스포린의 단백뇨 감소 효과가 당뇨병성 신병증에서도 관찰되는지를 알아봄과 동시에, 세극막 관련 단백의 하나인 P-cadherin의 발현 변화와의 연관성에 대해 연구하고자 하였다.

방 법: 24마리의 Sprague-Dawley 백서를 대조군 (8마리), 당뇨군

(8마리), 그리고 싸이클로스포린 (1.5 mg/kg/day, 피하투여) 처치 당뇨군 (8마리)으로 나누어 사육한 뒤, 당뇨 유발 6주 후에 분리한 사구체를 이용하여 calcineurin A-α, calcineurin A-β, P-cadherin, TGF-β_1 mRNA와 단백 발현의 변화를 각각 RT-PCR과 Western blot을 이용하여 관찰하였으며, 조직내 P-cadherin의 발현 변화는 면역형광 염색법을 이용하여 관찰하였다. 또한, 불멸 생쥐 족세포 (immortalized mouse podocytes)를 정상 포도당군 (5.6 mM), 정상 포도당+만니톨군 (24.4 mM), 정상 포도당+싸이클로스포린(10^{-8} M) 처치군, 정상 포도당+ TGF-β_1 (10 ng/ml) 처치군, 고포도당군 (30 mM), 또는 고포도당+싸이클로스포린 (10^{-8} M) 처치군으로 나누어 24시간 배양한 후 RT-PCR과 Western blot을 시행하였다.

결 과:

1. 24시간 뇨알부민 배설은 대조군 (0.32±0.02 mg)에 비해 당뇨군 (1.28±0.11 mg)에서 의의있게 높았으며, 싸이클로스포린 처치 당뇨군 (0.62±0.18 mg)에서는 당뇨군에 비해 뇨알부민 배설 증가가 유의하게 억제되었다 ($p<0.05$).
2. 사구체내 calcineurin A-α mRNA와 단백의 발현은 당뇨군에서 대조군에 비해 각각 2.1배, 2.2배 증가되었으나 ($p<0.05$), calcineurin A-β mRNA와 단백의 발현은 두 군 사이에 유의한 차이가 없었다.
3. 사구체내 P-cadherin mRNA와 단백의 발현은 당뇨군에서 대조군에 비해 각각 40.2%, 56.1% 감소되었으며 ($p<0.01$), 싸이클로스포린 처치 당뇨군에서는 이러한 P-cadherin의 발현 감소가 의미있게 억제되었다 ($p<0.05$).

4. 사구체내 TGF-β_1 mRNA와 단백의 발현은 당뇨군에서 대조군에 비해 각각 2.1배, 1.7배 증가되었으며, 싸이클로스포린 처치 당뇨군에서는 이러한 TGF-β_1의 발현 증가가 의의있게 억제되었다 ($p<0.05$).
5. 면역형광 염색 결과 사구체내 P-cadherin 단백의 발현은 족세포에 국한되었고, 대조군에 비해 당뇨군에서 유의하게 감소되어 있었으며 ($p<0.05$), 싸이클로스포린 처치 당뇨군에서는 P-cadherin 단백의 감소가 의미있게 회복되었다.
6. Calcineurin A-α mRNA와 단백의 발현은 정상 포도당군에 비해 고포도당으로 자극한 족세포군에서 각각 2.2배, 1.8배 증가되었으며, TGF-β_1을 처치한 정상 포도당군에서도 각각 2.3배, 1.7배 증가되었다 ($p<0.05$). 이에 반해 calcineurin A-β 발현은 모든 군 간에 유의한 차이가 없었다.
7. 고포도당은 배양 족세포의 P-cadherin mRNA와 단백 발현을 각각 50.4%, 66.4% 감소시켰으며, TGF-β_1으로 처치한 족세포군에서도 정상 포도당군에 비해 각각 49.3%, 67.5% 감소되었다 ($p<0.05$). 고포도당+싸이클로스포린 처치군에서는 P-cadherin mRNA와 단백 발현의 감소가 의미있게 억제되었다 ($p<0.05$).
8. TGF-β_1 mRNA와 단백의 발현은 고포도당군에서 정상 포도당군에 비해 각각 2.4배, 1.8배 증가되었으며, 고포도당+싸이클로스포린 처치군에서는 이러한 TGF-β_1의 발현 증가가 의의있게 억제되었다 ($p<0.05$).

결 론: 이상의 결과로, 싸이클로스포린은 당뇨 백서에서 뇨알부민 배설을 유의하게 감소시켰으며, 이러한 효과는 P-cadherin의 발현 변화와 밀접한 관련이 있을 것으로 사료된다. 또한,

싸이클로스포린에 의한 P-cadherin 발현 감소의 회복은 당뇨 조건 하에서 관찰되는 TGF-β_1의 발현 증가가 싸이클로스포린에 의해 억제되기 때문으로 생각된다.

핵심되는 말: 당뇨병성 신병증, 단백뇨, 족세포, 싸이클로스포린, P-cadherin, TGF-β_1