Iranian Journal of Basic Medical Sciences

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IJBMS

The role of sirolimus in proteinuria in diabetic nephropathy rats

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Mar 16, 2017
Accepted: Sep 28, 2017

Keywords:
Diabetic nephropathies
mTOR protein
Podocytes
Proteinuria
Sirolimus

ABSTRACT

Objective(s): The aim of this study was to observe the impact of sirolimus on proteinuria in streptozotocin (STZ) induced diabetic rats.

Materials and Methods: Rats were given a single injection of STZ to induce diabetic rat model. Rats' 24 hr urine was collected to test, urinary and the kidney tissues were harvested at the 8th and 20th weeks, respectively. Podocyte morphological changes were examined by electron microscopy and the ZO-1, podocin expressions in kidneys were detected by immunohistochemistry; the protein levels of Raptor and pS6 were measured by Western blot assay.

Results: In the early stage of diabetic nephropathy (DN), sirolimus reduced the proteinuria significantly (P<0.05); but in the advanced stage of DN, sirolimus worsened proteinuria (P<0.05). Electron microscopy test suggested that sirolimus could reduce the injury of podocyte at the early DN, but increased the injury at the late DN podocyte. Immunohistochemistry results indicated that sirolimus increased the expressions of podocin and ZO-1 at the early DN (P<0.05), but reduced the expressionsof ZO-1 and podocin (P<0.05) at the advanced DN. In the different periods of DN, the expression levels of Raptor and pS6 in sirolimus-treated groups were significantly lower than in the DN control groups (P<0.05).

Conclusion: Sirolimus can reduce proteinuria and alleviate the early DN podocyte injury in diabetic rat model by inhibiting the activity of mTORC1; but in the advanced stage of DN, sirolimus can increase podocyte injury and urine protein level.

► *Please cite this article as:*

Wang J, Xu Z, Chen B, Zheng Sh, Xia P, Cai Y. The role of sirolimus in proteinuria in diabetic nephropathy rats. Iran J Basic Med Sci 2017; 20:1339-1344. doi: 10.22038/IJBMS.2017.9618

Introduction

Nephrotoxicity of calcineurin inhibitors (CNI) is the leading cause of long-term graft dysfunction after renal transplantation. Therefore, alternative drugs are being explored. Recently, mammalian target of rapamycin (mTOR) receptor inhibitors (such as sirolimus and its derivatives everolimus) were widely used instead of CNI after transplantation. The mTOR therapy exhibited promising results such as improved and delayed deterioration of renal function (1).

The mTOR inhibitor sirolimus (SRL, also known as rapamycin) used to be reported as having no "nephro-toxicity", but the recent studies have found that SRL may increase proteinuria after transplantation (2, 3). Protein-uria worsened in patients when they converted CNI to sirolimus, especially in the patients who already had proteinuria before the conversion (4). Moreover, as reported, high concentration of SRL in the dose range of therapy may lead to renal lesions such as focal segmental glomerulosclerosis, along with massive proteinuria (5). However, it is noteworthy that, treatment with early conversion (post-operation, 3~6 months) from CNI to SRL or direct mTOR receptor inhibitors treatment after transplantation would decline the incidence of proteinuria and urine protein levels (1, 6). Therefore, early conversion is recommen-ded in the early stages after renal transplantation or in patients whose urine protein level is less than 500–800 mg in 24 hr (7). However, the effects of SRL on renal transplantation patients with different proteinuria levels remain unclear. Kim *et al.* found a significant interplay between SRL and CNI on the expression of the selected podocyte proteins in mouse podocytes. This might explain the higher incidence of proteinuria by CNI/SRL combination in clinical settings (8), and there is also a study that suggests mTOR inhibitors affect podocyte integrity with respect to podocyte proteins, cytoskeleton, inflamma-tion, and apoptosis (9). Besides, our previous study demonstrated that SRL treatment worsened proteinuria in the BSA-induced rat model, mainly through affecting the podocyte (10).

Podocyte injury is a classic marker of diabetic kidney damage, and rats in different stages of diabetic nephropathy (DN) exhibit different proteinuria levels (11). Susztak *et al.* claimed that glucose-induced ROS production initiates podocyte apoptosis and podocyte depletion *in vitro* and *in vivo* and suggest that podocyte apoptosis/depletion represents a novel early pathomechanism(s) leading to DN in murine type 1 and type 2 diabetic models (12). Early DN manifests kidney swelling, glomerular perfusion, microalbuminuria, and podocyte framework is basically normal; however, glomerular atrophy, interstitial fibrosis, proteinuria, and

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podocyte damage were shown at the late stages of DN (11, 13), and there is data that provides evidence for an association between podocyte loss and albumin excretion rate (14).

Therefore, we constructed the diabetic rat model to imitate patients with different levels of proteinuria after renal transplantation and tried to explore the mechanism of the effects of SRL on DN.

Materials and Methods

Animal studies

All animal procedures were carried out in accordance with international guidelines and approved by Wenzhou Medical University Animal Policy and Welfare Committee. Total of 37 male Sprague-Dawley rats at 8 weeks of age (body weight, 200~220 g) were obtained from Animal Experiment Center of Wenzhou Medical University and allowed to acclimate for 1 week. All rats were housed in plastic cages at 24~26°C and 55% humidity relatively with 12 hr: 12 hr light-dark cycles and free access to standard laboratory rodent chow and water before the experiment. The animals' body weights and food intake were measured weekly.

8 rats served as control. 29 rats were starved for 16 hr and injected once into the tail vein with STZ (55 mg/kg, Sigma-Aldrich, America) in sodium citrate buffer (1 mg/kg). After 72 hr, blood glucose was obtained by tail snipping. Rats with a blood glucose level≥16.7 mmol/l thrice were considered diabetic. For the experiment, the rats received insulin in the form of daily long-acting insulin (2~4 units, Lantus, Aventis) injections. The measurements were obtained while plasma glucose concentration was maintained at approximately 16~30 mmol/l under steady-state conditions.

Normal and diabetic rats were divided into five groups. Group I: Normal control group (n=8). Group II: Early diabetic nephropathy control group（DN) received saline solution for 8 weeks after being diabetic (2 ml/kg/day, n=8). Group III: The rats received sirolimus (Wyeth, America) for 4 weeks after 4 weeks being diabetic (2 ml/kg/day, n=8). Group IV: Advanced Diabetic nephropathy control group（DN) received saline solution for 20 weeks after being diabetic (2 ml/kg/day, n=6). Group V: The rats received sirolimus for 4 weeks after 16 weeks being diabetic (2 ml/kg/day, n=7). Urinary protein excretions were measured by our hospital's laboratory according to the manufacturer's instructions by collecting urine over a 24 hr period using metabolic cages at the 8th and 20th weeks (rats were fastened, but allowed water *ad libitum*).

Rats were anesthetized with chloral hydrate injected intraperitoneally (IP). Both kidneys were removed from renal capsules in an ice-brine bath. Kidney specimens were processed as previously described (15). Briefly, small renal tissue sections were obtained from renal cortex.

Histological examination

The kidney samples were fixed in 4% poly formaldehyde, and the tissue sections were stained with hematoxylin and eosin stains (HE). We examined the slides under light micro-scope (200×). For electron microscopy, some of the kidney tissues were fixed in 2.5% glutaraldehyde, some were fixed in 4% paraformaldehyde for immunohistochemistry. The rest of the samples were stored in liquid nitrogen for Western blot analysis later.

Immunohistochemistry

Tissue sections (3 μm) were rehydrated in 3% H_2O_2 at 37°C for 5~10 min and then blocked with 5% goat serum/PBS for 30 min. Rabbit polyclonal anti-ZO-1 (diluted 1:500; Santa Cruz Biotechnology) and rabbit anti-podocin polyclonal antibody (diluted 1:500; Santa Cruz Biotechnology) were applied to tissue sections according to the manufacturer's instructions. Each immunohistochemical assay included negative controls replacing the primary antibody with diluted primary antibody. After washing three times with TBS (5 min each), sections were incubated with HRP-conjugated anti-rabbit IgG secondary antibody (Amersham, Arlington Heights, IL) for 1 hr at 37°C. 50 μl of sigma-3,3′-diaminobenzidine substrate solution was added to sections and incubated for 10 min at room temperature (RT), rinsed thrice with PBS and counterstained with Mayer's hematoxylin for 6~10 min, dehydrated, cleared, and mounted in Entellan.

Linear and granular colored speckles (brown color) were defined as positive reactions of ZO-1 and podocin, respectively. Sections of each rat were quantitated by microscopic examination in randomly selected (n=10) high-power lens (x400) with the computer program, Image-Pro Plus 3.0 (Media Cybernetics, Silver Spring, MD).

ZO-1 can be expressed in renal tubular epithelium, while podocin is normally expressed only in glomeruli. Therefore, the positive areas of glomeruli from the inner renal cortex (area of interest, AOI) were selected for area measurements to analyze mean density (MD, MD=IOD sum/Area sum), it stands for the expression levels of ZO-1 or podocin protein in the positive part of the site).

Western blot assay

Western blot assays were performed as described previously (16). Briefly, renal tissues were homogenized in a lysis buffer and the supernatants were collected by centrifugation at 12,000 × g and 4 °C. After determining the total protein concentration, twenty micrograms of protein per specimen were run on 10% SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. After blocking with non-fat milk for 1 hr at RT, the membranes were incubated with different primary antibodies

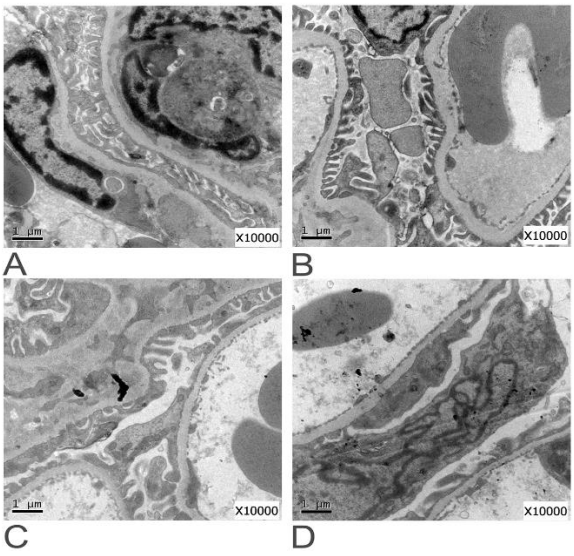

Figure 1. (A) Podocyte foot process fusion was seen in early diabetic rats. (B) Sirolimus reduced foot process fusion in early diabetic rats. (C) In advanced diabetic rats, foot process fusion increased. (D) Sirolimus aggravated the foot process fusion in advanced diabetic rats

overnight at 4 °C, washed with tris-buffered saline containing 0.05% Tween 20 and incubated with secondary horseradish peroxidase-conjugated antibody for 1 hr at RT. Antigen-antibody complexes were then visualized using an enhanced chemiluminescence kit (Amersham, Piscataway, NJ, USA), and the intensity of the protein bands was quantified using Quantity One software (version 4.6.2, Bio-Rad, USA). The primary antibodies against Raptor were purchased from Abcam (Cambridge, MA, USA), anti-Phospho-S6 was purchased from Cell Signaling Technology (Beverly, MA, USA) and β-actin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Statistical analyses

All experiments were performed at least thrice. Data were presented as means±SEM, and statistical comparison among multiple groups was performed by two-way analysis of variance (ANOVA) followed by Scheffe's *post hoc* test. When two independent sets of data were compared, the unpaired t-test was used. Differences between groups were considered statistically significant at $P<0.05$. All statistical calculations were performed using SPSS (ver. 17.0, SPSS Inc, Chicago, IL, USA)

Results

Sirolimus treatment reduced proteinuria at the early diabetic nephropathy but increased it at the late diabetic nephropathy

To analyze effects of sirolimus on diabetic rats, 24 hr urine protein levels were monitored during the early stages of diabetes. The urine protein contents from non-diabetic, diabetic, and sirolimus-treated diabetic rats were analyzed. The results showed that urine protein levels were higher in diabetic and sirolimus-treated diabetic rats than in non-diabetic rats after 8 weeks of diabetes, and sirolimus treatment significantly reduced urine protein levels as compared with untreated diabetic rats (Figure 1C).

To analyze the effects of sirolimus on the late stages of diabetes, urine protein levels were analyzed after 20 weeks of diabetes. The results showed that sirolimus treatment increased urine protein levels after 20 weeks of diabetes in rats (Figure 1F). These data indicate that sirolimus has opposite effects on urine protein accumulation at the early and late stages of diabetes.

Sirolimus reduced podocytes damage at the early stages of diabetic nephropathy while enhancing the damage at the late stages of diabetic nephropathy

Podocytes are cells in the Bowman's capsule in the kidneys that wrap around the capillaries of the glomerulus, and whose damage is the typical symptom of DN. Therefore, podocytes damages are observed at the early stage (8 weeks) of DN (Figure 1A). The results showed that sirolimus treatment reduce the podocytes damage and its barrier function to protect protein leakage (Figure 1B). At the late stage (20 weeks), foot process effacement was more obvious in sirolimus treatment group compared to the DN group (Figure 1D, E).

Sirolimus effects on podocin in podocytes

Podocin is located on slit diaphragm of podocytes, it can reflect the degree of podocytes damages (17,18). To study podocin's expression patterns in kidney, histochemical assay was performed by using non-diabetic, diabetic, and sirolimus-treated diabetic rat renal samples. After 8 weeks of diabetes, podocin levels were obviously lower in diabetic and sirolimus-treated diabetic groups than in the non-diabetic group. However, sirolimus treatment significantly increased podocin expressions compared to the untreated group after 8 weeks (Figure 2A).

After 20 weeks, sirolimus treatment obviously reduced podocin expressions (Figure 2B), suggesting that sirolimus reduced podocytes damages at the early stage of DN, but it has opposite effects at the late stage.

Sirolimus effect on ZO-1 protein in podocytes

ZO-1 protein represents a tight connection between podocyte and junction barrier as well as permeability function (19). In non-diabetic rat renal tissues, ZO-1 protein is along the glomerular capillary loops and tubular epithelium membrane with uniform linear distribution. At the 8th week of diabetes, ZO-1 protein levels were lower in diabetic and sirolimus-treated diabetic group than in the non-diabetic group.

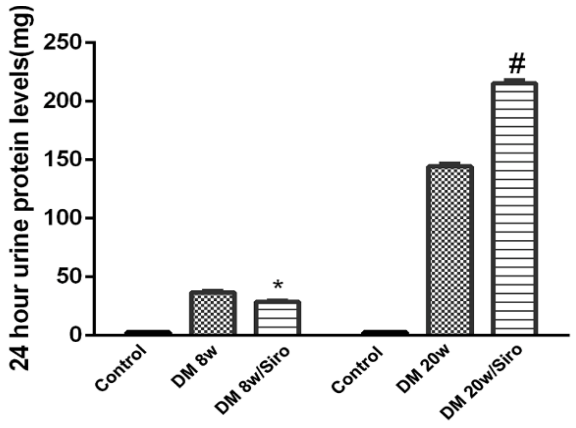

Figure 2. 24 hr urine protein levels. *P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 8 weeks; #P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 20 weeks

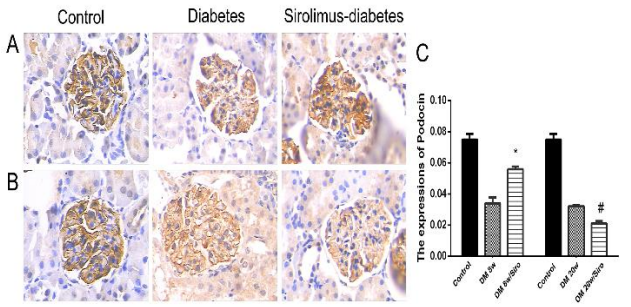

Figure 3. (A) Immunohistochemical staining of podocin in diabetic rats' kidneys of 8 weeks. (B) Immunohistochemical staining of podocin in diabetic rats' kidneys of 20 weeks. (C) Podocin expression levels of all the groups; *P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 8 weeks; #P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 20 weeks

However, sirolimus treatment significantly increased ZO-1 expression compared to untreated group (Figure 3A).

At the 20th week of diabetes, sirolimus treatment obviously reduced ZO-1 levels (Figure 3B) suggesting that sirolimus reduced podocytes damages on barrier function at the early stage of DN, but it has opposite effects to enhance the damages at the late stage.

Sirolimus effects on mTORC activity at the different stages of diabetic nephropathy

mTORC comprises mTORC1 and mTORC2, and Raptor is a specific component of mTORC1. Sirolimus has been known to mainly inhibiting mTORC1 activity (20, 21). S6 is a downstream molecule of mTOR signaling, and it is activated by phosphorylation (22). At the early stage of diabetes (8 weeks), sirolimus treatment significantly reduced the levels of Raptor and Phospho-S6 (pS6) compared to the untreated group (Figure 4).

At the late stage of diabetes (20 weeks), the expressions of Raptor and pS6 were analyzed after sirolimus medication. The results showed that sirolimus treatment obviously reduced Raptor and pS6 levels, indicating that sirolimus inhibits mTORC1 activity at different stages of diabetes in rats (Figure 5).

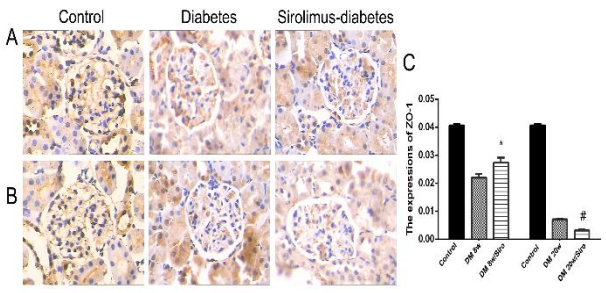

Figure 4. (A) Immunohistochemical staining of ZO-1 in 8 week diabetic rats' kidneys. (B) Immunohistochemical staining of ZO-1 in 20 week diabetic rats' kidneys. (C) ZO-1 expression levels of all groups; *P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 8 weeks; #P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 20 weeks

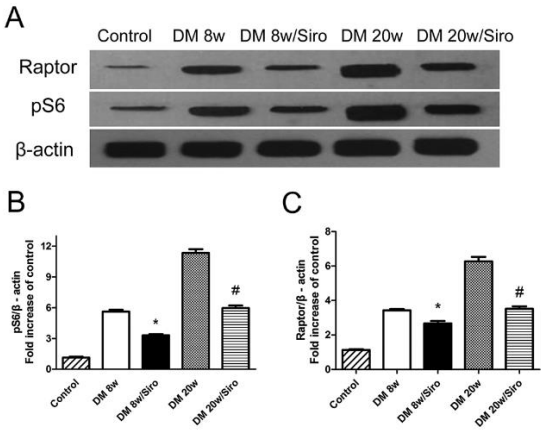

Figure 5. Western blot analysis of Raptor and pS6 expressions in each group; *P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 8 weeks; #P<0.05, sirolimus-treated group compared with untreated group in diabetic rats of 20 weeks

Discussion

The injury of glomerulus and loss of glomerular function are the main causes of CKD and would finally lead to ESRD. The podocytes in glomerulus are most susceptible to injury, the damages of which are associated with proteinuria and renal failure. Li *et al's* study indicated that baseline renal function, proteinuria, post-liver transplanta- tion, and diabetes are important for assessing the protective role of SRL in renal dysfunction (23). In our study, proteinuria level increased significantly in DN groups compared to the control group at 8 weeks; glomerular sclerosis and massive proteinuria were observed at 20 weeks. We found that sirolimus could reduce proteinuria in early DN rats, inhibit podocyte hypertrophy, and alleviate podocyte injury, but aggravated proteinuria in late DN rats.

Sirolimus, an mTOR receptor inhibitor, regulates cell growth, metabolic balance, proliferation, and apoptosis mainly through the PI-3K (PDK1) -Akt-mTORC1 intracellular kinase pathways. Sirolimus can cause proteinuria in humans and animal models (24-26), but there is also evidence that sirolimus can ameliorate renal function (27, 28). The mechanism of this phenomenon remains unclear, our results also showed this paradox.

There are two distinct mTOR complexes, rapamycin-sensitive mTOR complex1 (mTORC1) which includes Raptor, mLST8, and FK-BP12, and the rapamycin-insensitive mTORC2 which includes Rictor, mLST8, and Sin1 (11). There's report that inhibition of mTORC1 could cause proteinuria in renal transplant patients (29), use of sirolimus in patients with chronic allograft nephropathy could increase the risk for proteinuria (30); and there's evidence that both mTORC1 and mTORC2 are needed for podocyte development and podocyte maintenance, and mTOR function in podocytes is essential for the integrity of the filtration barrier (31).

An important feature of early DN is high perfusion, high pressure, and high filtration. Our study found that renal structure exhibited hypertrophy of podo-cytes, which may result in increased permeability, and also found that the expression of podocyte markers (ZO-1 and podocin) were reduced, indicating that podocytes were damaged. Finally, these changes led to microalbuminuria (32). At early DN, the expression of mTORC1 Raptor and its downstream effector molecular pS6 were significantly increased, indicating that the activation of the mTORC1 pathway could lead to microalbuminuria. We found sirolimus could inhibit the kinase activity of mTORC1, thereby significantly reduce urine protein level at early DN, which was consistent with the previous report (33). Podocyte proliferation did not occur at mature glomerular, so there must be volume expansion in podocytes to accommodate the high glomerular perfusion mainly through the regulation of mTORC1 activity (16, 34-35). So sirolimus could reduce podocyte hypertrophy, permeability, and proteinuria through inhibition of mTORC1 activity.

In the advanced stage of DN, renal lesions are mainly characterized by tubular atrophy, interstitial fibrosis, and podocytes loss. Podocytes are terminally differentiated epithelial cells lacking the ability to proliferate. In recent years, there are reports that parietal epithelial cells (PECs) of Bowman's capsule, which are also known as podocyte precursor cells, can repair this damage (7, 10). PECs' homeostasis could be influenced by regulating the activity of mTOR (36). Because sirolimus can regulate metabolic balance during various cell proliferation and apoptosis processes, we hypothesized that sirolimus aggravating filtration barrier damage and proteinuria may be through inhibiting the activity of mTORC1 in PECs and hampering PECs' differentiation into podocytes to repair damage at the late DN. The mechanism of this phenomenon needs to be studied further.

Conclusion

We found that sirolimus treatment can reduce proteinuria at the early stage of DN; however, sirolimus increased proteinuria at the advanced stage of DN, which is mainly through the regulation of mTORC1 activity. The study provides a theoretical basis for correctly timed use of sirolimus and reducing its damage to the kidneys in renal transplant patients.

Acknowledgment

This project was supported by grants from Natural Science Foundation of Zhejiang province (LY17H100005), Science and Technology Department of Wenzhou, China (Y20130199).

Conflicts of interest

All the authors declare no financial and non-financial conflicts of interest.

References

1. Lebranchu Y, Thierry A, Toupance O, Westeel PF, Etienne I, Thervet E, *et al*. Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. Am J Transplant 2009; 9:1115-1123.

2. Franco-Esteve A, Tordera D, de la Sen ML, Jiménez L, Mas P, Muñoz C, *et al*. mTOR inhibitor monotherapy. A good treatment choice in renal transplantation? Nefrologia 2012; 32:631-638.

3. van den Akker JM, Wetzels JF, Hoitsma AJ. Proteinuria following conversion from azathioprine to sirolimus in renal transplant recipients. Kidney Int 2006; 70:1355-1357.

4. Aliabadi AZ, Pohanka E, Seebacher G, Dunkler D, Kammerstätter D, Wolner E, *et al*. Development of proteinuria after switch to sirolimus-based immunosuppression in long-term cardiac transplant patients. Am J Transplant 2008; 8:854-861.

5. Letavernier E, Bruneval P, Mandet C, Duong Van Huyen JP, Péraldi MN, Helal I, *et al*. High sirolimus levels may induce

focal segmental glomerulosclerosis de novo. Clin J Am Soc Nephrol 2007; 2:326-333.
6. Izzedine H, Brocheriou I, Frances C. Post-transplantation proteinuria and sirolimus. N Engl J Med 2005; 353:2088-2089.
7. Kaplan B, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. Transplant Rev 2014; 28:126-133.
8. Kim BS, Cho Y, Lee H, Joo DJ, Huh KH, Kim MS, *et al*. Comparative proteomic analysis of rapamycin versus cyclosporine combination treatment in mouse podocytes. Transplant Proc 2016; 48:1297-1301.
9. Müller-Krebs S, Weber L, Tsobaneli J, Kihm LP, Reiser J, Zeier M, et al. Cellular effects of everolimus and sirolimus on podocytes. PLoS One 2013; 8:e80340.
10. Cai Y, Chen Y, Zheng S, Chen B, Yang Y, Xia P. Sirolimus damages podocytes in rats with protein overload nephropathy. J Nephrol 2011; 24:307-312.
11. Menini S, Iacobini C, Oddi G, Ricci C, Simonelli P, Fallucca S, *et al*. Increased glomerular cell (podocyte) apoptosis in rats with streptozotocin-induced diabetes mellitus: role in the development of diabetic glomerular disease. Diabetologia 2007; 50:2591-2599.
12. Susztak K, Raff AC, Schiffer M, Böttinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes 2006; 55:225-233.
13. Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology 2007; 12:261-266.
14. White KE, Bilous RW, Marshall SM, El Nahas M, Remuzzi G, Piras G, *et al*. Podocyte number in normotensive type 1 diabetic patients with albuminuria. Diabetes 2002; 51:3083-3089.
15. Attia DM, Feron O, Goldschmeding R, Radermakers LH, Vaziri ND, Boer P, *et al*. Hypercholesterolemia in rats induces podocyte stress and decreases renal cortical nitric oxide synthesis via an angiotensin II type 1 receptor-sensitive mechanism. J Am Soc Nephrol 2004; 15:949-957.
16. Liu XD, Zhang LY, Zhu TC, Zhang RF, Wang SL, Bao Y. Overexpression of miR-34c inhibits high glucose-induced apoptosis in podocytes by targeting Notch signaling pathways. Int J Clin Exp Pathol 2015; 8:4525-4534.
17. Roselli S, Gribouval O, Boute N, Sich M, Benessy F, Attié T, *et al*. Podocin localizes in the kidney to the slit diaphragm area. Am J Pathol 2002; 160:131-139.
18. Michaud JL, Lemieux LI, Dubé M, Vanderhyden BC, Robertson SJ, Kennedy CR. Focal and segmental glomerulosclerosis in mice with podocyte-specific expression of mutant alpha-actinin-4. J Am Soc Nephrol J 2003; 14:1200-1211.
19. Rincon-Choles H, Vasylyeva TL, Pergola PE, Bhandari B, Bhandari K, Zhang JH, *et al*. ZO-1 expression and phosphorylation in diabetic nephropathy. Diabetes 2006; 55:894-900.
20. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006; 124:471-484.
21. Kim DH, Sabatini DM. Raptor and mTOR: subunits of a nutrient-sensitive complex. Curr Top Microbiol Immunol 2004; 279:259-270.
22. Thomas G. The S6 kinase signaling pathway in the control of development and growth. Biol Res 2002; 35:305-313.
23. Li LC, Hsu CN, Lin CC, Cheng YF, Hu TH, Chen DW, *et al*. Proteinuria and baseline renal function predict mortality and renal outcomes after sirolimus therapy in liver transplantation recipients. BMC Gastroenterol 2017; 17:58.
24. Mjörnstedt L, Sørensen SS, von Zur Mühlen B, Jespersen B, Hansen JM, Bistrup C, *et al*. Improved renal function after early conversion from a calcineurin inhibitor to everolimus: a randomized trial in kidney transplantation. Am J Transplant 2012; 12:2744-2753.
25. Mandelbrot DA, Alberú J, Barama A, Marder BA, Silva HT Jr, Flechner SM, et al. Effect of ramipril on urinary protein excretion in maintenance renal transplant patients converted to sirolimus. Am J Transplant 2015; 15:3174-3184.
26. Taveira-DaSilva AM, Jones AM, Julien-Williams PA, Stylianou M, Moss J. Retrospective review of combined sirolimus and simvastatin therapy in lymphangioleiomyomatosis. Chest 2015; 147:180-187.
27. Franco A, Más-Serrano P, Perez Contreras J, Jiménez L, Rodriguez D, Olivares J. Mammalian target of rapamycin inhibitor monotherapy: efficacy in renal transplantation. Transplant Proc 2015; 47:2364-2367.
28. Jun H, Kim MG, Jung CW. Clinical advantages including medication adherence with conversion to once-daily advagraf and sirolimuscombination in stable kidney recipients. Int J Clin Pharmacol Ther 2016; 54(2):81-6.
29. Amer H, Cosio FG. Significance and management of proteinuria in kidney transplant recipients. J Am Soc Nephrol 2009; 20:2490-2492.
30. Letavernier E, Bruneval P, Mandet C, Duong Van Huyen JP, Péraldi MN, Helal I, *et al*. High sirolimus levels may induce focal segmental glomerulosclerosis de novo. Clin J Am Soc Nephrol 2007; 2:326-333.
31. Godel M, Hartleben B, Herbach N, Liu S, Zschiedrich S, Lu S, *et al*. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. J Clin Invest 2011; 121:2197-2209.
32. Jefferson JA, Shankland SJ, Pichler RH. Proteinuria in diabetic kidney disease: a mechanistic viewpoint. Kidney Int 2008; 74:22-36.
33. Xiao T, Guan X, Nie L, Wang S, Sun L, He T, *et al*. Rapamycin promotes podocyte autophagy and ameliorates renal injury in diabetic mice. Mol Cell Biochem 2014; 394:145-154.
34. Polak P, Hall MN. mTOR and the control of whole body metabolism. Curr Opin Cell Biol 2009; 21:209-218.
35. Mori H, Inoki K, Masutani K, Wakabayashi Y, Komai K, Nakagawa R, *et al*. The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. Biochem Biophys Res Commun 2009; 384:471-475.
36. Hamatani H, Hiromura K, Sakairi T, Takahashi S, Watanabe M, Maeshima A, *et al*. Expression of a novel stress-inducible protein, sestrin 2, in rat glomerular parietal epithelial cells. Am J Physiol Renal Physiol 2014; 307:F708-F717.