Differential effect of statins on diabetic nephropathy in db/db mice

YUKINORI TAMURA[1,3], TOSHINORI MURAYAMA[1], MANABU MINAMI[1], MASAYUKI YOKODE[1] and HIDENORI ARAI[2]

Departments of [1]Clinical Innovative Medicine and [2]Human Health Sciences, Kyoto University Graduate School of Medicine, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Received June 3, 2011; Accepted July 6, 2011

DOI: 10.3892/ijmm.2011.769

Abstract. Recent studies suggest a potential benefit of the lipid-lowering medication in the treatment of chronic kidney disease (CKD) such as diabetic nephropathy. Although statins have been widely used to lower serum cholesterol levels, the effect of these drugs on diabetic nephropathy has not been fully elucidated. In the present study, therefore, we addressed the role of different kinds of statins on diabetic nephropathy in db/db mice. Mice were fed with a standard diet with 0.005% (w/w) of pitavastatin, rosuvastatin, and pravastatin for 8 weeks starting from 8 weeks of age. The treatment with statins did not affect the food intake, body weight gain, adiposity, or blood pressure in db/db mice. Treatment with statins also had no effect on plasma lipid levels. In terms of the effect on albuminuria, pitavastatin and rosuvastatin reduced the urinary excretion of albumin by 60 and 40%, respectively, but not pravastatin, suggesting the effect of these two drugs on diabetic nephropathy. Furthermore, pitavastatin and rosuvastatin improved glomerular hypertrophy. All statins treatment improved insulin resistance. In addition, rosuvastatin and pravastatin treatment reduced oxidative stress measured by urinary 8-OHdG level, whereas the statins had no effect on the inflammatory response in the kidney of db/db mice. These results are not consistent with the renoprotective effect of statins. In conclusion, our data suggest that pitavastatin and rosuvastatin can improve diabetic nephropathy through the suppression of glomerular hypertrophy, independent of lipid-lowering or anti-oxidative effects.

Introduction

Diabetic nephropathy is one of the most common forms of chronic kidney disease (CKD) and the most frequent cause of mortality in patients with diabetes (1,2). The number of people affected by diabetic nephropathy or who need renal replacement is steadily increasing (3). Therefore, the establishment of therapeutic strategies for diabetic nephropathy is needed. Diabetic nephropathy results from complex interactions between genetic, metabolic, and hemodynamic factors, and can be characterized by mesangial expansion followed by glomerulosclerosis and a decline in renal function. The development of glomerulosclerosis in diabetes mellitus is always preceded by persistent albuminuria and glomerular hypertrophy (2). Therefore, these two manifestations could be promising therapeutic targets for the treatment of diabetic nephropathy.

3-Hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors (statins) are widely used for diabetic patients to reduce their cardiovascular risk (4). Statins also have renoprotective actions and have been shown to reduce albuminuria in both experimental and clinical diabetic renal disease (5-8). Some of these benefits may be due to lipid lowering, since lipid levels are strongly associated with the development and progression of diabetic kidney disease (9,10). On the other hand, statins have a range of lipid-independent actions on cell proliferation, inflammation, and oxidative stress (11,12), which may impact the development and progression of renal damage in diabetes. These pleiotropic effects have been suggested to contribute to the renoprotective effect of statins. However, the precise mechanisms of the renoprotective effects are not fully understood. In addition, whether different statins have the same effect on diabetic nephropathy is not well known.

In this study, we addressed the role of various statins, such as pitavastatin, rosuvastatin, and pravastatin on the development of diabetic nephropathy in db/db mice.

Materials and methods

Materials. Pravastatin and rosuvastatin were provided by Daiichi Sankyo Co., Ltd. and pitavastatin was provided by Kowa Pharmaceutical Co., Ltd.

Animal procedure and experimental design. Male db/db mice (n=24) and their lean control db/m (n=6) mice were obtained from Charles River at 6 weeks of age. The mice were fed with normal chow without additional supplementation (non-treated group) or with chow supplemented with 0.005% (w/w) pravastatin, pitavastatin or rosuvastatin for 8 weeks starting from 8

Correspondence to: Dr Hidenori Arai, Department of Human Health Sciences, Kyoto University Graduate School of Medicine, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
E-mail: harai@kuhp.kyoto-u.ac.jp

Present address: [3]Department of Physiology and Regenerative Medicine, Kinki University School of Medicine, 377-2 Ohno-higashi, Osakasayama, Osaka 589-8551, Japan

Key words: statin, diabetic nephropathy, albuminuria, pleiotropic action

Table I. Characteristics of db/m and db/db mice treated with or without statins.

		db/db			
	db/m	Con	Pra	Pit	Ros
Body weight (g)	32.5±0.40	53.1±3.90	51.2±5.1	50.3±3.80	51.3±5.70
Liver weight (g)	1.15±0.29	2.99±0.41	2.78±0.54	2.55±0.36	3.19±0.85
eWAT weight (g)	0.37±0.05	3.23±0.25	3.08±0.59	3.01±0.41	3.10±0.62
Kidney weight (g)	0.31±0.07	0.50±0.02	0.51±0.06	0.42±0.01[a]	0.41±0.03[a]
Food intake (g/day)	3.82±0.33	7.45±2.43	7.15±0.72	7.70±1.65	7.04±1.58
SBP (mmHg)	NA	113.2±11.6	113.5±3.00	109.5±9.50	114.6±5.60

Con, control; Pra, pravastatin; Pit, pitavastatin; Ros, rosuvastatin; eWAT, epididymal white adipose tissue; SBP, systolic blood pressure. Results are expressed as mean ± SD (n=6 in each group). [a]P<0.05 vs. Con.

weeks of age. Animals had access to food and water *ad libitum* and were maintained on a 12-h light/dark cycle. All animal experiments were conducted according to the Guidelines for Animal Experiments at Kyoto University.

Analysis of metabolic parameter. Plasma glucose concentration was measured with a Glutest Ace (Sanwa Kagaku Kenkyusho Co., Ltd.). Plasma insulin concentration was measured with an insulin assay kit (Morinaga Institute of Biological Science). Plasma cholesterol and triglyceride levels were respectively measured with the Cholesterol E and Triglyceride E tests (Wako Pure Chemical Industries, Ltd.).

Measurement of urinary albumin and creatinine. Urinary albumin and creatinine were measured at 16 weeks of age from 24-h collection samples from mice housed in individual metabolic cages. During the urine collection, the mice were allowed free access to food and water. Albumin concentration in the urine was measured by Albuwell (Exocell). Urinary creatinine was measured with a Hitachi Mode 736 analyzer (Hitachi). The urinary albumin concentration was adjusted by the urinary creatinine concentration.

Measurement of urinary oxidative stress. Urinary 8-OHdG concentrations were measured at 16 weeks of age using a competitive enzyme-linked immunosorbent assay kit (8-OHdG Check, Japan Institute for the Control of Aging). Urinary 8-OHdG excretion was expressed as the total amount excreted in 24 h.

Quantitative real-time PCR. Total-RNA was extracted from frozen kidney tissue (50 mg) at 16 weeks of age using an RNeasy mini kit (Qiagen). The cDNA was synthesized from total-RNA using SuperScript III (Invitrogen). Real-time PCR was performed on an ABI PRISM 7900 using the SYBR-Green PCR Master Mix (Applied Biosystems). Primer sets were as follows: tumor necrosis factor (TNF)-α forward, 5'-CCCAGA CCCTCACACTCAGATC-3' and reverse, 5'-GCCACTCCAG CTGCTCCTC-3'; β-actin forward, 5'-TACCACAGGCATTG TGATGG-3' and reverse, 5'-TTTGATGTCACGCACGAT TT-3'. The mRNA levels were normalized relative to the amount of β-actin mRNA and expressed in arbitrary units.

Measurement of glomerular size. The mice were euthanized at 16 weeks of age. The kidneys were rapidly fixed in 10% formaldehyde, and embedded in paraffin. Paraffin sections were cut at 3 μm. For measurement of the glomerular size, paraffin sections were stained with hematoxylin and eosin. The size of the glomerular surface area was measured using the Image-Pro Plus software version 3.0.1 (Media Cybernetics, Inc.).

Statistical analysis. Data are expressed as the mean ± SD. Multiple comparisons among the groups were conducted by one-way analysis of variance with Fisher's PLSD test for post hoc analysis. P-values of <0.05 were considered significant.

Results

Effect of statin treatment on body weight, adiposity and systolic blood pressure. In db/db mice fed with a standard diet for 8 weeks starting at 8 weeks of age, body weight, epididymal white adipose tissue (eWAT) weight, liver weight were increased compared to those of db/m mice. Treatment with statins had no effect on body weight, food intake, liver weight and eWAT weight in db/db mice (Table I). In addition, there was no difference in systolic blood pressure between statin-treated and non-treated db/db mice.

Effect of statin treatment on renal function in db/db mice. Because albuminuria reflects renal function (13), we measured the urinary excretion of albumin in normal chow-fed db/db mice at 16 weeks of age. Urinary excretion of albumin was markedly increased in db/db mice compared with db/m mice (Fig. 1). Pitavastatin, rosuvastatin, but not pravastatin improved albuminuria in db/db mice. Kidney weights in pitavastatin- and rosuvastatin-treated db/db mice were reduced compared with non-treated db/db mice (Table I). These data suggest that pitavastatin and rosuvastatin treatment improves renal function in db/db mice.

Effect of statin treatment on plasma lipid level in db/db mice. To clarify the mechanism by which statins ameliorated renal function, we first examined the effect of statin treatment on lipid metabolism in db/db mice. Plasma triglyceride and total cholesterol level were increased in non-treated db/db mice compared with db/m mice (Fig. 2A and B). On the other hand,

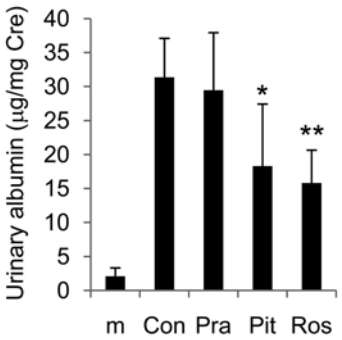

Figure 1. Effect of statins on renal function in db/db mice. The graph shows the urinary excretion of albumin in db/m mice (m), non-treated (Con), pravastatin-treated (Pra), pitavastatin-treated (Pit) and rosuvastatin-treated (Ros) db/db mice. Results are expressed as mean ± SD. *P<0.05, **P<0.01 vs. non-treated db/db mice (n=6 in each group).

statin treatment had no effect on plasma lipid levels in db/db mice (Fig. 2A and B), suggesting that the renoprotective effect of statins is independent of their lipid-lowering action.

Effect of statin treatment on insulin resistance in db/db mice. It has been reported that the development of insulin resistance contributes to renal dysfunction (14). Therefore, we next examined the effect of statin treatment on glucose metabolism in db/db mice. Blood glucose level, plasma insulin level, and HOMA-IR were markedly increased in db/db mice compared with db/m mice, indicating an increase in insulin resistance (Fig. 2C-E). Although statin treatment had no effect on plasma glucose, all statins reduced plasma insulin levels, resulting in a decrease in HOMA-IR (Fig. 2C-E). The data suggest that statin treatment improves insulin resistance.

Because hypoadiponectinemia is associated with the development of insulin resistance and kidney disease (15), we examined the effect of statin treatment on plasma adiponectin levels in db/db mice. In non-treated db/db mice, plasma adiponectin levels were decreased compared with db/m mice. Meanwhile, statin treatment had no effect on plasma adiponectin level in db/db mice (Fig. 2F).

Effect of statin treatment on the renal inflammation in db/db mice. Accumulating evidence now indicates that inflammatory mechanisms play a significant role in the development and progression of diabetic nephropathy. Especially, TNF-α is a pleiotropic inflammatory cytokine and has been shown to cause enhanced albumin permeability (16). Therefore, we next examined the effect of statin treatment on inflammation in the kidney of db/db mice. The expression of TNF-α mRNA was increased in the kidney of db/db mice compared with that of db/m mice, whereas statin treatment had no effect on its expression in db/db mice (Fig. 3A). These data suggest that statins had no effect on the inflammatory response in the kidneys of db/db mice.

Effect of statin treatment on the oxidative stress in db/db mice. To examine the effect of statin treatment on oxidative stress, we measured urinary 8-OHdG concentrations in db/db mice. Urinary 8-OHdG levels in non-treated db/db mice were significantly higher than those in db/m mice. Pravastatin and rosuvastatin reduced urinary 8-OHdG levels in db/db mice, whereas pitavastatin had no effect on oxidative stress despite detecting the amelioration of albuminuria (Fig. 3B).

Effect of statin treatment on glomerular hypertrophy in db/db mice. Glomerular hypertrophy is a hallmark in diabetic

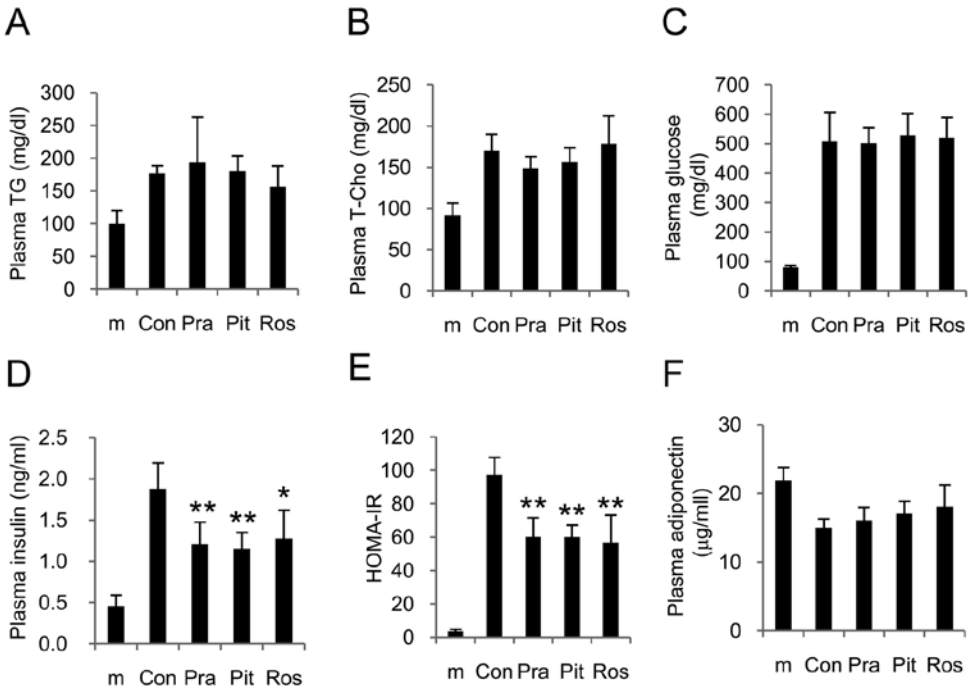

Figure 2. Effect of statins on lipid and glucose metabolism in db/db mice. (A) Plasma triglyceride (TG), (B) total cholesterol (T-Cho), (C) glucose, (D) insulin, (E) HOMA-IR and (F) adiponectin levels in db/m mice (m), non-treated (Con), pravastatin-treated (Pra), pitavastatin-treated (Pit) and rosuvastatin-treated (Ros) db/db mice. Results are expressed as mean ± SD. *P<0.05, **P<0.01 vs. non-treated db/db mice (n=6 in each group).

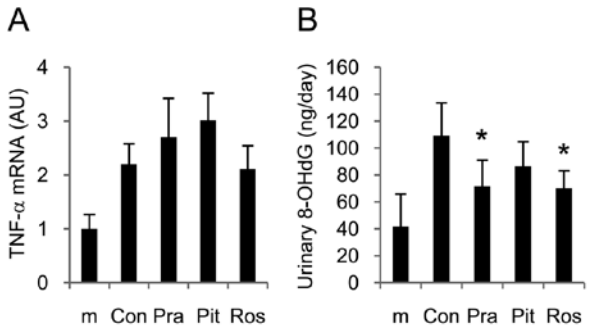

Figure 3. Effect of statins on renal inflammation and oxidative stress in db/db mice. (A) Expression of TNF-α mRNA in whole kidney and (B) urinary 8-OHdG levels in db/m mice (m), non-treated (Con), pravastatin-treated (Pra), pitavastatin-treated (Pit) and rosuvastatin-treated (Ros) db/db mice. Results are expressed as mean ± SD. *P<0.05 vs. non-treated db/db mice (n=6 in each group).

nephropathy along with albuminuria. Therefore, we assessed the glomerular hypertrophy in db/db mice and the effect of statins by measuring the glomerular surface area. Mean glomerular surface area size in db/db mice was increased compared with db/m mice. Pitavastatin and rosuvastatin treatment, but not pravastatin treatment, suppressed the glomerular hypertrophy as well as urinary excretion of albumin in db/db mice (Fig. 4).

Discussion

In the present study, we showed that pitavastatin and rosuvastatin treatment improved albuminuria and suppressed glomerular hypertrophy, independent of its lipid-lowering and anti-oxidative effect in db/db mice.

In CKD patients, there is an increase in total cholesterol and LDL levels (17). The level of cholesterol is directly correlated with the degree of albuminuria (18), suggesting that hyperlipidemia is associated with the development of CKD such as diabetic nephropathy. In fact, lipid-lowering therapy by statin has been successful to the amelioration of renal function in patients with diabetic nephropathy (19,20). However, the present study and other animal studies showed that statin treatment significantly improved renal function without affecting the plasma lipid profile (5,8,21). Therefore, the renoprotective effect of statins may be mainly caused by its pleiotropic action rather than their lipid-lowering action.

Insulin resistance is associated with the development of renal dysfunction in type 2 diabetes. It has been shown that insulin resistance correlates with the onset of microalbuminuria in patients with type 2 diabetes as well as in nondiabetic subjects (14). Several studies showed that amelioration of insulin resistance resulted in a restoration of renal function (22-24). Statin also has an ability to ameliorate insulin resistance. Takagi *et al* (25) reported that pravastatin treatment improved insulin resistance through the increase in plasma adiponectin levels in db/db mice. In the present study, we also observed that all statin treatment improved insulin resistance detected by the reduction of HOMA-IR, while adiponectin was not altered by statin treatment in db/db mice. However, this amelioration was not consistent with the renoprotective effects of statins in db/db mice.

Oxidative stress and inflammation are also far more prevalent in CKD patients than in normal subjects (26). In the present study, we also observed the elevation of oxidative stress and inflammation in the kidneys of db/db mice compared with that of lean control mice. Renal disease is associated with a graded increase in oxidative stress markers even in early CKD (27). This oxidative stress can accelerate renal injury progression. In addition, inflammatory markers such as C reactive protein and cytokines increase with renal function deterioration suggesting that CKD is a low-grade inflammatory process (28). Therefore, the agents which have anti-oxidative and anti-inflammatory action have been attracted as a therapeutic strategy for renal dysfunction (29). Anti-oxidative and anti-inflammatory actions are also major pleiotropic effects of statins (12). Several reports have shown that these actions of statins contribute to their renoprotective effects (5,30,31). In the present study, we also observed that pravastatin and rosuvastatin suppressed oxidative stress in db/db mice as well as these reports, whereas we could not detect the anti-inflammatory effect of statins in the kidneys of db/db mice. Pitavastatin had no effect on oxidative stress, despite the presence of the restored renal function in db/db mice. This result suggests that the anti-oxidant action of statins is not primarily responsible for their renoprotective effect.

In the present study, we observed a correlation between the renoprotective effects of statins and their suppressive effect

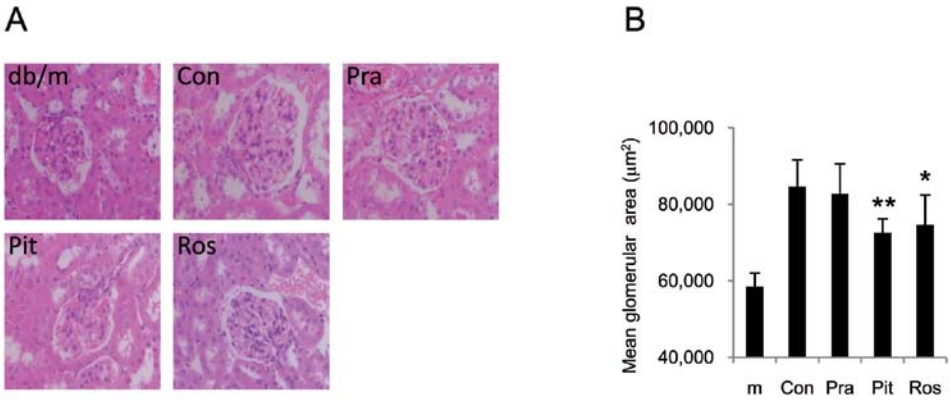

Figure 4. Effects of statins on the glomerular hypertrophy in db/db mice. (A) H&E staining of glomeruli (magnification, x200) and (B) mean glomerular surface area of db/m mice (m), non-treated (Con), pravastatin-treated (Pra), pitavastatin-treated (Pit) and rosuvastatin-treated (Ros) db/db mice. The mean area of fifty glomeruli per mouse was analyzed. Results are expressed as mean ± SD. *P<0.05, **P<0.01 vs. non-treated db/db mice (n=6 in each group).

of glomerular hypertrophy in db/db mice. The glomerular morphological changes in diabetic nephropathy are characterized primarily by mesangial expansion and glomerular based membrane (GBM) thickening. It has been reported that the dysregulated cell cycle by the increased inhibitor of cyclin dependent kinase (such as p21 and p27) contributes to these morphological changes and renal dysfunction (32,33). Pleiotropic effects of statins on the cell cycle are well known (12). Furthermore, Danesh *et al* (34) reported that statin treatment normalized the cell cycle through the suppression of p21 expression in high glucose-stimulated mesangial cells. In the present study, pleiotropic effects of statin on the cell cycle thus might improve glomerular hypertrophy and albuminuria. However, further study is required to clarify the effect of statins in glomerular hypertrophy and renal dysfunction.

In conclusion, we have shown the effects of various statins on diabetic nephropathy in db/db mice. Our study suggests that its renoprotective effect is mainly dependent on suppressing the glomerular hypertrophy, independent of its lipid-lowering or anti-oxidative effects, and there may be differences in the renoprotective ability between various statins.

Acknowledgements

We thank Dr Takeshi Matsubara (Kyoto University) for helpful discussion and critical reading of our manuscript. We also thank Nami Sawada (Kyoto University) for excellent technical assistance. This study was supported by the Takeda Science Foundation.

References

1. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM and Goetz FC: Structural-functional relationships in diabetic nephropathy. J Clin Invest 74: 1143-1155, 1984.
2. Nagai K, Arai H, Yanagita M, *et al*: Growth arrest-specific gene 6 is involved in glomerular hypertrophy in the early stage of diabetic nephropathy. J Biol Chem 278: 18229-18234, 2003.
3. Coresh J, Selvin E, Stevens LA, *et al*: Prevalence of chronic kidney disease in the United States. JAMA 298: 2038-2047, 2007.
4. Athyros VG, Mitsiou EK, Tziomalos K, Karagiannis A and Mikhailidis DP: Impact of managing atherogenic dyslipidemia on cardiovascular outcome across different stages of diabetic nephropathy. Expert Opin Pharmacother 11: 723-730, 2010.
5. Fujii M, Inoguchi T, Maeda Y, *et al*: Pitavastatin ameliorates albuminuria and renal mesangial expansion by downregulating NOX4 in db/db mice. Kidney Int 72: 473-480, 2007.
6. Kolavennu V, Zeng L, Peng H, Wang Y and Danesh FR: Targeting of RhoA/ROCK signaling ameliorates progression of diabetic nephropathy independent of glucose control. Diabetes 57: 714-723, 2008.
7. Nakamura T, Ushiyama C, Hirokawa K, Osada S, Shimada N and Koide H: Effect of cerivastatin on urinary albumin excretion and plasma endothelin-1 concentrations in type 2 diabetes patients with microalbuminuria and dyslipidemia. Am J Nephrol 21: 449-454, 2001.
8. Ota T, Takamura T, Ando H, Nohara E, Yamashita H and Kobayashi K: Preventive effect of cerivastatin on diabetic nephropathy through suppression of glomerular macrophage recruitment in a rat model. Diabetologia 46: 843-851, 2003.
9. Rosario RF and Prabhakar S: Lipids and diabetic nephropathy. Curr Diab Rep 6: 455-462, 2006.
10. Thomas MC, Rosengard-Barlund M, Mills V, *et al*: Serum lipids and the progression of nephropathy in type 1 diabetes. Diabetes Care 29: 317-322, 2006.
11. Campus G, Salem A, Sacco G, Maida C, Cagetti MG and Tonolo G: Clinical effects of mechanical periodontal therapy in type 2 diabetic patients. Diabetes Res Clin Pract 75: 368-369, 2007.
12. Haslinger-Loffler B: Multiple effects of HMG-CoA reductase inhibitors (statins) besides their lipid-lowering function. Kidney Int 74: 553-555, 2008.
13. Dronavalli S, Duka I and Bakris GL: The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab 4: 444-452, 2008.
14. Jauregui A, Mintz DH, Mundel P and Fornoni A: Role of altered insulin signaling pathways in the pathogenesis of podocyte malfunction and microalbuminuria. Curr Opin Nephrol Hypertens 18: 539-545, 2009.
15. Tesauro M, Canale MP, Rodia G, *et al*: Metabolic syndrome, chronic kidney, and cardiovascular diseases: role of adipokines. Cardiol Res Pract 2011: 653182, 2011.
16. Navarro-Gonzalez JF and Mora-Fernandez C: The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 19: 433-442, 2008.
17. Kwan BC, Beddhu S, Kronenberg F and Cheung AK: Does statin therapy improve cardiovascular outcomes in patients with type 2 diabetes receiving hemodialysis? Nat Clin Pract Nephrol 2: 76-77, 2006.
18. Kaysen GA, Gambertoglio J, Felts J and Hutchison FN: Albumin synthesis, albuminuria and hyperlipemia in nephrotic patients. Kidney Int 31: 1368-1376, 1987.
19. Tonolo G, Velussi M, Brocco E, *et al*: Simvastatin maintains steady patterns of GFR and improves AER and expression of slit diaphragm proteins in type II diabetes. Kidney Int 70: 177-186, 2006.
20. Sharp Collaborative Group: Study of Heart and Renal Protection (SHARP): randomized trial to assess the effects of lowering low-density lipoprotein cholesterol among 9,438 patients with chronic kidney disease. Am Heart J 160: 785-794 e10, 2010.
21. Usui H, Shikata K, Matsuda M, *et al*: HMG-CoA reductase inhibitor ameliorates diabetic nephropathy by its pleiotropic effects in rats. Nephrol Dial Transplant 18: 265-272, 2003.
22. Okada T, Wada J, Hida K, *et al*: Thiazolidinediones ameliorate diabetic nephropathy via cell cycle-dependent mechanisms. Diabetes 55: 1666-1677, 2006.
23. Baylis C, Atzpodien EA, Freshour G and Engels K: Peroxisome proliferator-activated receptor [gamma] agonist provides superior renal protection versus angiotensin-converting enzyme inhibition in a rat model of type 2 diabetes with obesity. J Pharmacol Exp Ther 307: 854-860, 2003.
24. Miyazaki Y, Cersosimo E, Triplitt C and DeFronzo RA: Rosiglitazone decreases albuminuria in type 2 diabetic patients. Kidney Int 72: 1367-1373, 2007.
25. Takagi T, Matsuda M, Abe M, *et al*: Effect of pravastatin on the development of diabetes and adiponectin production. Atherosclerosis 196: 114-121, 2008.
26. Schiffrin EL, Lipman ML and Mann JF: Chronic kidney disease: effects on the cardiovascular system. Circulation 116: 85-97, 2007.
27. Krane V and Wanner C: The metabolic burden of diabetes and dyslipidaemia in chronic kidney disease. Nephrol Dial Transplant 17 (Suppl 11): S23-S27, 2002.
28. Mora C and Navarro JF: Inflammation and diabetic nephropathy. Curr Diab Rep 6: 463-468, 2006.
29. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C and Zoccali C: Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant 18: 1272-1280, 2003.
30. Zhou MS, Schuman IH, Jaimes EA and Raij L: Renoprotection by statins is linked to a decrease in renal oxidative stress, TGF-beta, and fibronectin with concomitant increase in nitric oxide bioavailability. Am J Physiol Renal Physiol 295: F53-59, 2008.
31. Diepeveen SH, Verhoeven GW, Van Der Palen J, *et al*: Effects of atorvastatin and vitamin E on lipoproteins and oxidative stress in dialysis patients: a randomised-controlled trial. J Intern Med 257: 438-445, 2005.
32. Al-Douahji M, Brugarolas J, Brown PA, Stehman-Breen CO, Alpers CE and Shankland SJ: The cyclin kinase inhibitor p21WAF1/CIP1 is required for glomerular hypertrophy in experimental diabetic nephropathy. Kidney Int 56: 1691-1699, 1999.
33. Awazu M, Omori S, Ishikura K, Hida M and Fujita H: The lack of cyclin kinase inhibitor p27(Kip1) ameliorates progression of diabetic nephropathy. J Am Soc Nephrol 14: 699-708, 2003.
34. Danesh FR, Sadeghi MM, Amro N, *et al*: 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors prevent high glucose-induced proliferation of mesangial cells via modulation of Rho GTPase/p21 signaling pathway: implications for diabetic nephropathy. Proc Natl Acad Sci USA 99: 8301-8305, 2002.