Does combined peroxisome proliferator-activated receptors-agonist and pravastatin therapy attenuate the onset of diabetes-induced experimental nephropathy?

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ABSTRACT

الأهداف: التحقيق في الآثار المتجمعة للروسيجليتازون و البرافاستاتين على وظائف الكلى فى الفئران المصابة بالقصور الكلوى السكرى الناجم عن الستربتوزوتوسين (DN).

الطريقة: أجريت هذه الدراسة في بيت الحيوانات بمستشفى الملك خالد الجامعي، الرياض، المملكة العربية السعودية من أغسطس 2013م إلى فبراير 2014م. تم توزيع 50 فأراً على مجموعات منها مجموعة ضابطة و مجموعة مصابة بداء السكري كان منها ما تلقى سالين، روسيجليتازون، برافاستاتين، أو روسيجليتازون + برافاستاتين معاً لمدة شهرين. كان وزنها ما بين 230-250 جم، و أعمارها ما بين 18-20 أسبوع. بعد نهاية التجربة، تم قياس نسبة صفاء الكرياتينين ونسبة زلال البول إلى نسبة الكرياتينين (ACR). تم تحليل عينات الدم للترانسفيرين،الهيموغلوبين الغليكوزيلاتي (HbA1c)، الدهون، عامل النخر الورمي ألفا (TNF-α) عامل الالتصاق البين خلوى - 1 (ICAM-1)، وبيروكسيد الدهون.

النتائج: علاج الروسيجليتازون زاد نسبة صفاء الكرياتينين والترانسفيرين في البلازما، وخفض نسبة زلال البول ACR، و نسبة HbA1c، والبلازما ICAM-1، TNF-α، ومستويات بيروكسيد الدهون في الدم دون التأثير على مستوى الدهون في الدم المتغير. أنتج علاج برافاستاتين نتائج مماثلة و ضبط مستوى تغيير الدهون. كان مزج روسيجليتازون وبرافاستاتين معا أكثر فعالية في التقليل من حدوث التلف الكلوي الناتج عن السكري مقارنة مع المجموعة التي تم علاجها بإحدى العلاجين.

الخاتمة: تفيد الدراسة إمكانية استخدام الروسيجليتازون أو البرافاستين لعلاج أو تقليل حدوث التلف الكلوي الناتج عن السكري، خاصة عند إعطائهما معا.

Objectives: To investigate the combined effects of rosiglitazone and pravastatin on renal functions in early streptozotocin induced diabetic nephropathy (DN).

Methods: This study was carried out at King Khalid University Hospital Animal House, Riyadh, Saudi Arabia from August 2013 to February 2014. Fifty male Wistar rats were assigned to normal control rats and diabetic rats that received saline, rosiglitazone, pravastatin, or rosiglitazone+pravastatin for 2 months. Their weight range was 230-250 gm, and age range was from 18-20 weeks. At the end of experiment, creatinine clearance, and urinary albumin to creatinine ratio (ACR) were measured. Blood samples were analyzed for transferrin, glycosylated hemoglobin (HbA1c), lipid profile, tumor necrosis factor-alpha (TNF-α), intercellular adhesion molecule-1 (ICAM-1), and lipid peroxide.

Results: Rosiglitazone treatment increased creatinine clearance and plasma transferrin, and decreased urinary ACR, HbA1c, plasma TNF-α, ICAM-1, and serum lipid peroxide levels without affecting the altered lipid profile. Pravastatin treatment produced similar results and normalized the lipid alteration. The combination of rosiglitazone and pravastatin was more effective in attenuating the diabetes-induced nephropathy compared with treatment with either drug alone.

Conclusion: The combination strategy of rosiglitazone and pravastatin may provide a potential synergistic renoprotective effect against DN by improving renal functions and reducing indices of DN.

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Diabetic nephropathy (DN) is one of the major complications of uncontrolled and chronic diabetes mellitus (DM), and is the most common cause of progressive renal damage and end stage renal failure in diabetic patients.[1] Approximately 20-30% diabetic patients develop signs of nephropathy.[2] Diabetic nephropathy is associated with renal structural alterations, such as glomerular basement membrane thickening, mesangial cell expansion, and podocyte loss.[3] Currently, DN has considerable impact on society in the areas of social economy and public health.[4] Moreover, the current therapeutic strategies for treating DN are insufficient as most diabetic patients continue to show progressive renal damage. Therefore, developing new therapeutic interventions to prevent, or even attenuate the progression of DN is one of the targets of the current research interest. Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors of nuclear hormone receptor superfamily, which comprises of 3 members: PPARα, PPARβ, and PPARγ.[5] The PPARγ is expressed mainly in mesangial, endothelial, and vascular smooth muscle cells.[5] Thiazolidinediones, such as rosiglitazone is well-known PPARγ agonists employed as insulin sensitizing antidiabetic agents.[6] Treatment with rosiglitazone (PPARγ agonist) has been demonstrated to possess renoprotective effect as it reduces albuminuria, prevents renal endothelial dysfunction, and reduced over expression of intracellular adhesion molecule-1 (ICAM-1) in glomerular mesangial cells in patients with DN.[7] Three-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely used for diabetic patients to reduce their cardiovascular risks.[8] Statins also have renoprotective actions, and have been shown to reduce albuminuria in both experimental and clinical diabetic renal disease.[9] Some of these benefits may be due to lipid lowering, since DM-associated lipid alteration and dyslipidemia could significantly contribute to the development of DN.[10] On the other hand, statins have a range of lipid-independent actions on cell proliferation/apoptosis, oxidative stress, and inflammation,[11] which may impact the development and progression of renal damage in diabetes. We have previously shown that both pravastatin and 12/15 lipooxygenase pathway inhibitor (nordihydroguaiaretic acid) had favorable effects on renal functions of diabetes-induced nephropathy in rats.[12] The present study aimed to assess the effect of combination therapy of rosiglitazone (PPARγ agonist) and pravastatin (HMG-CoA reductase inhibitor) on renal functions by determining creatinine clearance (CC), urinary albumin to creatinine ratio, levels of transferrin, tumor necrosis factor-alpha (TNF-α), ICAM-1 and lipid peroxide in streptozotocin-induced DN.

Disclosure. This research project was supported by a grant from the Research Center of the Female Scientific and Medical Colleges, Deanship of Scientific Research, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Methods. This study was carried out on 50 male Wistar rats at King Khalid University Hospital Animal House, Riyadh, Kingdom of Saudi Arabia from August 2013 to February 2014. The rat's weight range was from 230-250 gm, and age range was from 18-20 weeks. To induce diabetes, rats were injected with streptozotocin (STZ) 65 mg/kg intraperitonealy.[13] The STZ was dissolved in 0.1 M citrate buffer (pH 4.5) immediately before use. Fasting tail-vein blood glucose level was measured by an Accu-Chek Active System glucometer (Roche Diagnostics, Mannheim, Germany) on the third day after STZ injection. Rats with fasting blood glucose more than 300 mg/dL were considered diabetic. Rats were assigned to 5 groups (10 rats/group): Group 1 - included normal control rats. Groups 2, 3, 4, and 5 - included diabetic rats receiving saline, rosiglitazone, pravastatin, or both rosiglitazone and pravastatin. Rosiglitazone was given at a dose of 5 mg/kg per day in drinking water for 2 months.[14] Pravastatin was gavaged at a dose of 0.4 mg/kg in a dilution of normal saline daily for 2 months.[15] All drugs and chemicals were supplied by Sigma Laboratories (St. Louis, MO, USA). To assess the diabetes-induced nephropathy, animals were kept in metabolic cages separately at the end of drug treatment, and 24 hour urine samples were collected. Urinary albumin and creatinine excretion were measured. In order to adjust for the variability of urine collection, the urinary albumin to creatinine ratio (ACR) was measured in each sample.[16] All animals were fasted overnight but allowed free access to water. A blood sample was withdrawn by the retro orbital sinus under mild ether anesthesia, and the samples were collected in EDTA and plain tubes, then centrifuged. Plasma and serum were separated and stored at -70°C until completion of the analysis. Experiments were conducted in accordance with Institutional Review Board at King Khalid University Hospital according to NIH Guiding Principles in the Care and Use of Animals.

To measure changes overtime, a baseline measurement for the control group and the intervention groups was taken, and compared with the measurements obtained 2 months after completion of the experiment. Blood samples were analyzed for glycosylated hemoglobin (HbA1c). Serum creatinine[17] and lipid profile (triglycerides, cholesterol, low density lipoprotein cholesterol [LDLC], high density lipoprotein cholesterol [HDLC]) were measured using colorimetric method.[18] Plasma samples were used for measuring the levels of transferrin, using Rat Transferrin ELISA kit, catalog No. E-25TX (Immunology Consultants Laboratory Inc., Portland, OR, USA),[19] TNF-α using Rat TNF-α Quantikine Solid Phase Sandwich ELISA Kit, catalog No. RTA00 (R&D Systems Inc., USA, Minneapolis, MN, USA), and a soluble form of intercellular adhesion molecule-1 (ICAM-1) using Rat ICAM-1/CD54 Quantikine ELISA Kit, catalog No. RIC100 (R&D Systems Inc., MN, USA).[20] Serum lipid peroxide was measured using Lipid Peroxidation (MDA) colorimetric Assay Kit, catalog No. MAK085 (Sigma-Aldrich Co., St. Louis, MO, USA).[21]

Statistical analysis. The Statistical Package for Social Sciences for Windows version 21 (IBM Corp, Armonk, NY, USA) program was used for statistical analysis. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by one way analysis of variance (ANOVA) with 95% confidence intervals (CI) followed by Tukey's multiple comparison tests. Paired-sample T test was performed for comparing baseline measurements with those measured 2 months after completion of the experiment. Differences were considered to be statistically significant at $p<0.05$.

Results. Tables 1 & 2 show baseline measurements, which were compared with those taken 2 months after completion of the experiment in each group. Statistically significant changes were detected when comparing baseline measurements of each parameter to those taken 2 months after completing the experiment in pravastatin (Group 4), and rosiglitazone+pravastatin (Group 5) treated groups. Similar results were detected for rosiglitazone treated group, except no significant changes were detected between baseline measurement of lipid profile, and those taken after completing the experiment. At the end of the experiment, CC was significantly decreased in diabetic control rats compared with normal controls. Treatment with either rosiglitazone or pravastatin significantly increased CC compared with diabetic control rats. Combination therapy with rosiglitazone and pravastatin increased CC as compared with treatments with either drug alone (**Figure 1**). In addition, a significant increase in urinary ACR was found in diabetic control rats compared with normal control rats. Treatment with either rosiglitazone or pravastatin partially reduced urinary ACR in diabetic rats. Moreover, the combined therapy with rosiglitazone and pravastatin markedly reduced the urinary ACR compared with treatments with either drug alone (**Figure 2**).

Regarding plasma transferrin, it was significantly decreased at the end of the experiment in diabetic control rats compared with the normal control group. This decrease could be due to excessive loss of transferrin through the kidneys into the urine. Either rosiglitazone or pravastatin treatment significantly increased plasma transferrin compared with diabetic control rats. The combination therapy of rosiglitazone and pravastatin normalized plasma transferrin (**Table 3**). The significant increase in HbA1c at the end of the experiment was noted in diabetic control rats compared with normal rats. Treatment with pravastatin partially reduced the HbA1c level in diabetic rats. However, treatment with rosiglitazone markedly reduced HbA1c level in diabetic rats. In addition, the effect of pravastatin in partially reducing HbA1c level in diabetic rats was enhanced by its combination with rosiglitazone (**Table 3**). The increase in serum concentrations of cholesterol, triglycerides, LDLC, and consequent decrease in HDLC levels were detected in diabetic control rats after completing the experiment. Treatment with pravastatin significantly attenuated diabetes-induced alteration in the lipid profile. However, treatment with rosiglitazone did not affect the lipid alterations in diabetic rats, while the effect of pravastatin in attenuating lipid alterations in diabetic rats was enhanced by its combination with rosiglitazone (**Table 3**). Moreover, plasma TNF-α was significantly increased in diabetic control rats as compared to normal control group. Also, rosiglitazone or pravastatin treatment significantly decreased plasma TNF-α as compared to diabetic control rats, but it was significantly increased by rosiglitazone or pravastatin treatment as compared to normal controls and those rats treated by combined rosiglitazone and pravastatin treatment. So, combination therapy of rosiglitazone and pravastatin normalized plasma TNF-α (**Figure 3**).

Similar results were found for plasma ICAM-1 where it was significantly increased in diabetic control rats compared with the normal control group. Also, treatment with either rosiglitazone or pravastatin significantly decreased plasma ICAM-1 compared with diabetic control rats. Moreover, plasma ICAM-1 was significantly decreased by rosiglitazone and pravastatin

Table 1 - Mean values±standard deviation (SD) of creatinine clearance, urinary albumin to creatinine ratio (ACR), and lipid profile in controls (Group 1), diabetic controls (Group 2), rosiglitazone (Group 3), pravastatin (Group 4), and rosiglitazone+pravastatin (Group 5) treated groups (baseline, and 2 months after completion of the experiment).

Variables	Group 1		Group 2		Group 3		Group 4		Group 5	
	Baseline	After	Baseline	After	Baseline	After	Baseline	After	Baseline	After
Creatinine clearance										
Mean±SD	1.606±0.171	1.609±0.175	0.859±0.080	0.851±0.078	1.098±0.14	1.238±0.11	1.130±0.178	1.272±0.144	1.199±0.135	1.560±0.196
t	1.152		-3.207		12.332		3.667		10.580	
P-value	0.279		0.011*		0.000*		0.005*		0.000*	
ACR										
Mean±SD	10.5±2.273	10.7±2.791	230.3±28.58	235.6±27.69	234.8±28.15	154.1±10.19	233.6±24.50	149.7±11.01	236.8±24.458	117.8±5.432
t	0.557		2.776		-6.957		-7.708		-14.183	
P-value	0.591		0.022*		0.000*		0.000*		0.000*	
Cholesterol										
Mean±SD	50.115±2.551	50.884±2.231	67.765±3.187	68.665±2.914	65.612±2.051	66.005±1.96	65.104±2.34	57.078±2.267	64.012±1.856	52.845±1.904
t	1.450		2.083		0.874		-14.687		-10.694	
P-value	0.181		0.067		0.405		0.000*		0.000*	
Triglycerides										
Mean±SD	75.497±3.217	75.445±2.967	169.185±6.057	169.470±6.344	166.810±5.531	167.179±5.36	167.910±4.470	115.555±2.967	166.91±6.256	79.974±3.031
t	-0.154		0.525		1.780		-51.751		-35.495	
P-value	0.881		0.613		0.109		0.000*		0.000*	
LDLC										
Mean±SD	41.869±4.449	41.389±3.683	66.153±3.908	66.365±3.987	61.109±4.884	61.223±5.14	61.923±4.124	51.576±5.244	60.734±5.093	42.133±3.161
t	-0.495		1.643		0.636		-6.281		-12.735	
P-value	0.633		0.135		0.540		0.000*		0.000*	
HDLC										
Mean±SD	36.856±2.477	36.736±2.566	26.552±2.714	26.717±2.698	25.652±2.768	26.011±3.06	25.882±3.127	32.472±3.309	26.125±2.923	37.876±3.059
t	-0.376		-0.606		1.553		5.170		10.659	
P-value	0.720		0.560		0.155		0.001*		0.000*	

LDLC - low density lipoprotein cholesterol, HDLC - high density lipoprotein cholesterol. *significant values

Table 2 - Mean values ± standard deviation of glycosylated hemoglobin (HbA1C), transferrin, tumor necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM-1), and lipid peroxide in controls (Group 1), diabetic controls (Group 2), rosiglitazone (Group 3), pravastatin (Group 4), and rosiglitazone+pravastatin (Group 5) treated groups (baseline and 2 months after completion of the experiment). *significant values

Variables	Group 1		Group 2		Group 3		Group 4		Group 5	
	Baseline	After	Baseline	After	Baseline	After	Baseline	After	Baseline	After
HbA1C										
Mean±SD	3.91±0.40	3.89±0.39	6.82±0.49	6.95±0.56	6.89±0.42	4.22±0.49	6.92±0.49	5.31±0.44	6.89±0.49	3.98±0.64
t	-0.502		2.786		-15.258		-7.958		-10.545	
P-value	0.627		0.021*		0.000*		0.000*		0.000*	
Transferrin										
Mean±SD	1.66±0.15	1.65±0.13	0.99±0.06	0.98±0.06	0.99±0.05	1.36±0.08	0.99±0.05	1.37±0.08	0.99±0.05	1.55±0.12
t	-0.147		-4.583		19.510		20.054		14.104	
P-value	0.887		0.001*		0.000*		0.000*		0.000*	
TNF-α										
Mean±SD	14.73±0.66	14.73±0.60	22.74±1.66	22.96±1.57	22.15±2.09	18.82±0.59	22.81±1.48	18.77±0.51	23.20±1.87	14.96±1.34
t	0.054		1.606		-4.561		-9.179		-19.012	
P-value	0.958		0.143		0.001*		0.000*		0.000*	
ICAM										
Mean±SD	1.38±0.17	1.38±0.23	2.29±0.47	2.38±0.46	2.28±0.37	1.76±0.26	2.23±0.45	1.46±0.29	2.46±0.44	1.34±0.24
t	0.215		2.161		-3.249		-3.787		-6.733	
P-value	0.835		0.059		0.010*		0.004*		0.000*	
Lipid peroxide										
Mean±SD	3.10±0.62	3.065±0.64	8.11±0.33	8.14±0.38	7.96±0.48	5.12±0.48	7.93±0.57	5.23±0.35	7.95±0.49	2.99±0.41
t	-1.440		1.000		-14.119		-13.427		-20.199	
P-value	0.184		0.343		0.000*		0.000*		0.000*	

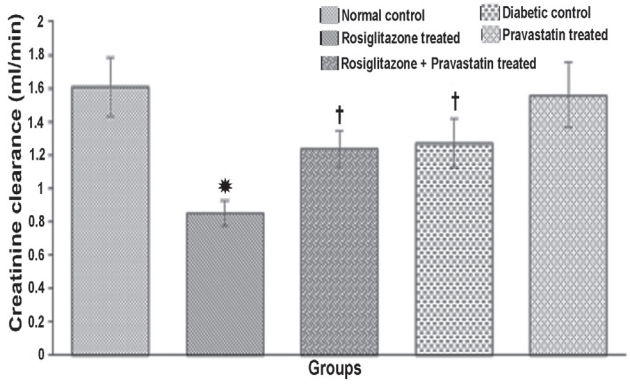

Figure 1 - Effect of rosiglitazone and pravastatin on creatinine clearance. All values are represented as mean ± standard deviation. **p*=0.000 versus all other groups, †*p*=0.000 versus normal control and rosiglitazone + pravastatin treated diabetic group.

combination treatment compared with those treated by rosiglitazone alone (*p*=0.024). However, no significant differences were detected between pravastatin therapies alone, or in combination with rosiglitazone (**Figure** 4). Diabetic control rats showed a marked increase in serum lipid peroxide level compared with normal rats. Treatment with either rosiglitazone or pravastatin partially attenuated the diabetes-induced increase in serum lipid peroxide level. Moreover, the combined therapy with rosiglitazone and pravastatin markedly attenuated the diabetes-induced increase in serum lipid peroxide level compared with treatments with either drug alone. As a result, a combination therapy of rosiglitazone and pravastatin normalized serum lipid peroxide as it normalized plasma TNF-α (**Figure 5**).

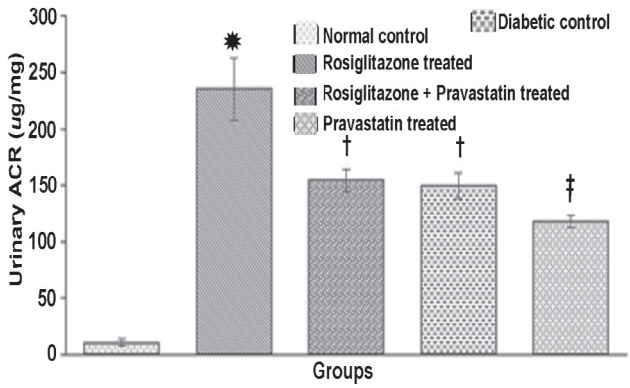

Figure 2 - Effect of rosiglitazone and pravastatin on urinary albumin to creatinine ratio (ACR). All values are represented as mean ± standard deviation. **p*=0.000 versus all other groups; †*p*=0.000 versus normal control and rosiglitazone + pravastatin treated diabetic group. ‡*p*=0.000 versus normal control.

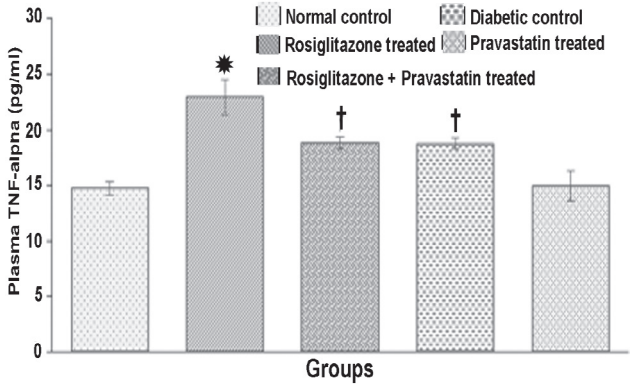

Figure 3 - Effect of rosiglitazone and pravastatin on plasma tumor necrosis factor (TNF)-α 2 months after completion of the experiment. All values are represented as mean ± standard deviation. **p*=0.000 versus all other groups; †*p*=0.000 versus normal control and rosiglitazone + pravastatin treated diabetic group.

Table 3 - Mean values ± standard deviation of transferrin, glycosylated hemoglobin and lipid profile in the studied groups 2 months after completion of the experiment.

Variables	Group 1 (Normal controls) (n=10 rats)	Group 2 (Diabetic control) (n=10 rats)	Group 3 (Rosiglitazone treated diabetics) (n=10 rats)	Group 4 (Pravastatin treated diabetics) (n=10 rats)	Group 5 (Rosiglitazone+Statin treated diabetics) (N=10 rats)	F value
Transferrin	1.652 ± 0.128	0.979 ± 0.060*	1.360 ± 0.080§	1.369 ± 0.083§	1.553 ± 0.124	68.406
HbA1c (%)	3.890 ± 0.387	6.950 ± 0.558*	4.220 ± 0.485	5.310 ± 0.441†	3.980 ± 0.639	64.403
Cholesterol (mg/dl)	50.88 ± 2.23	68.67 ± 2.91**	66.01 ± 1.96**	57.08 ± 2.27†	52.85 ± 1.90	119.714
Triglycerides (mg/dl)	75.45 ± 2.97	169.47 ± 6.34**	167.18 ± 5.36**	115.56 ± 2.97‡	79.97 ± 3.03	1078.956
LDLC (mg/dl)	41.39 ± 3.68	66.37 ± 3.99**	61.22 ± 5.14**	51.58 ± 5.24‡	42.13 ± 3.16	66.859
HDLC (mg/dl)	36.74 ± 2.57	26.72 ± 2.70**	26.01 ± 3.06**	32.47 ± 3.31‡	37.88 ± 3.06	34.716

Significance was considered at $p<0.05$, 95% confidence interval. HbA1c - glycosylated hemoglobin, LDLC - low density lipoprotein cholesterol, HDLC - high density lipoprotein cholesterol.**p*=0.000 compared with groups 1, 3, 4, 5, †*p*=0.000 compared with groups 1, 3, and 5, ‡*p*=0.000 compared with groups 1, and 5, §*p*=0.001 compared with groups 1 and 5, ***p*=0.000 compared with groups 1, 4, and 5

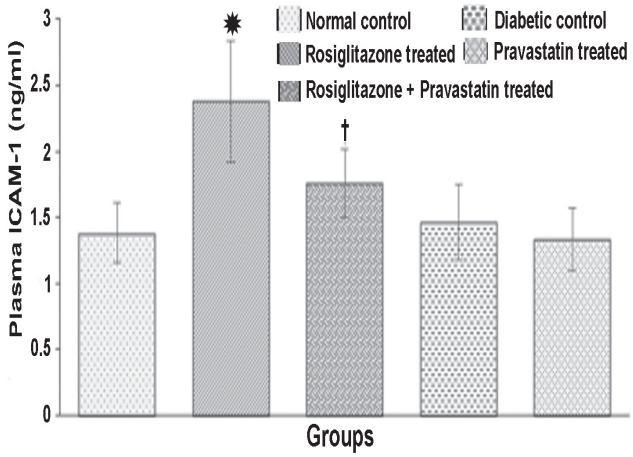

Figure 4 - Effect of rosiglitazone and pravastatin on plasma intercellular adhesion molecule (ICAM-1) 2 months after completion of the experiment. All values are represented as mean ± standard deviation. *p=0.000 versus all other groups; †p=0.024 versus rosiglitazone + pravastatin treated diabetic group.

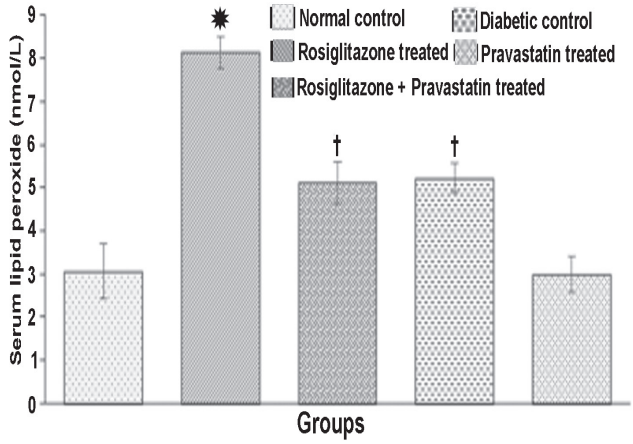

Figure 5 - Effect of rosiglitazone and pravastatin on serum lipid peroxide 2 months after completion of the experiment. All values are represented as mean ± standard deviation. *p=0.000 versus all other groups, †p=0.000 versus normal control and rosiglitazone + pravastatin treated diabetic group.

Discussion. The present study showed favorable effects of combined rosiglitazone and pravastatin therapy on renal function in diabetes-induced experimental nephropathy. Creatinine clearance was noted to decrease, while urinary ACR increased in diabetic control rats compared with normal rats. In addition, the diabetic rats showed decreased plasma transferrin and increased plasma TNF-α, ICAM-1, and serum lipid peroxide after 2 months. These changes indicate pathological changes in glomeruli that have been documented to be indices of DN. The pharmacological treatment with either rosiglitazone or pravastatin partially prevented the diabetes-induced nephropathy by increasing CC and plasma transferrin, attenuating the increase in ACR, and the alteration in lipid profile observed in diabetic rats. Furthermore, treatment with either rosiglitazone or pravastatin decreased plasma TNF-α, ICAM-1, and serum lipid peroxide levels. These effects were potentiated by combined rosiglitazone and pravastatin treatment.

Increased CC by rosiglitazone and pravastatin treatment indicates improvement of renal function. A meta-analysis clinical study supported the effectiveness of short term high-dose statin pretreatment for decreasing the level of serum creatinine.[22] Although the precise mechanism involved in the statin effect on extracellular matrix proteins at the cellular and molecular level is not known, it is suggested that statins lipid-independent effects on endothelial function via increasing NO upregulation, and reducing oxidative stress, and vascular inflammation. Thus, in the kidney, statins could also counteract inflammation by rising eNOS activity, and hence, NO bioavailability.[23] Treatment with rosiglitazone has been demonstrated to possess a renoprotective effect as it reduces albuminuria and prevents renal endothelial dysfunction in diabetic patients with nephropathy.[24] Rosiglitazone has been shown to prevent the development of DN by reducing podocyte loss, downregulating the expression of glomerular fibronectin, and inhibiting the accumulation of reactive oxygen species (ROS) in glomeruli of mice with DN.[25]

Our results of attenuation in the increase of urinary ACR by rosiglitazone treatment is consistent with the study of Lachin et al,[26] who reported a significant decrease of urinary ACR after a 3-month treatment of type 2 diabetic patients with rosiglitazone. Since albuminuria is a modifiable risk factor for the progression to end-stage renal disease, and a marker of endothelial dysfunction, the anti-proteinuric effect of rosiglitazone and pravastatin could be a significant advantage in reducing renal risk in diabetic patients. Follow-up studies showed that increased transferrin excretion predicted the development of microalbuminuria in diabetic patients. This provides evidence that urinary transferrin may be a more sensitive indicator of glomerular damage and DN than standard microalbuminuria.[27] In the present study, plasma transferrin decreased significantly in diabetic control rats, which may be due to its excessive loss through the kidneys into the urine. Attenuatation of the decrease in plasma transferrin by treatment with rosiglitazone and pravastatin, either alone or combined indicates improvement of renal function.

Uncontrolled hyperglycemia plays an important role in the induction and progression of DN by accelerating the process of renal advanced glycation end products formation, and generating ROS that damage the structure and affect the function of the diabetic kidney.[28] In this study, HbA1c levels (which reflect control of diabetes over the last 3 months) increased in diabetic control rats compared with normal controls. Rosiglitazone treatment of diabetic rats markedly reduced HbA1c level without altering the diabetes-induced elevated circulating lipids. Rosiglitazone has been shown to increase pancreatic beta-cell mass in an animal model of type 2 diabetes.[29] Moreover, the glucose-lowering action of rosiglitazone is partly related to improving insulin sensitivity, besides a direct protective effect on beta cells, stimulating the release and synthesis of insulin, and preventive effect on beta-cell apoptosis.[30]

Growing evidence suggests that the elevation in circulating lipids has been found to be an important predictor of renal function loss, and may contribute to the induction and progression of nephropathy. The circulating lipids are trapped by renal extracellular matrix molecules, where they undergo oxidization, and thus increase the generation of ROS, which may deteriorate the structure and function of the diabetic kidney.[31] In the present study, marked increases in serum cholesterol, triglycerides and LDLC, and consequent decrease in serum HDL levels were noted in diabetic rats. Rosiglitazone treatment of diabetic rats did not affect the altered lipid profile. This is consistent with the previous study of Arora et al.[32] However, treatment with pravastatin either alone, or in combination with rosiglitazone significantly attenuated diabetes-induced alteration in lipid levels. In fact, statins have multiple proposed 'pleiotropic' mechanisms of action that may affect progression of renal disease, independent of their lipid-lowering effect. Statins inhibit leukocyte and mesangial cell expression of inflammatory chemokines. Also, statins can inhibit the proliferation of mesangial, renal tubular, and vascular smooth muscle cells. Another potential benefit of statins is improvement of vascular hemodynamic responses through their effect on endothelial function in addition to their antioxidant effect.[12] Many studies pointed out the possibilities of PPAR-dependent renoprotective effects of statins. It has been shown that pravastatin pretreatment in carboplatin-administered mice considerably prevented the induction of renal dysfunction and apoptosis, and improved renal morphology, and survival by inducing the expression of PPARα.[33] Increasing evidence has implicated TNF-α as a major participant in the pathogenesis of kidney injury by promoting inflammation, apoptosis, accumulation of extracellular matrix, and damage the glomerular permeability barrier with the development of albuminuria. In addition, TNF-α directly induces the production of ROS in diverse cells, including mesangial cells, which results in the alterations of the barrier function of the glomerular capillary wall, and leads to enhanced albumin permeability.[34]

The ICAM-1 is a key adhesion molecule that mediates monocytes/macrophages infiltration into the diabetic kidney, and inducing the proliferation of the mesangial cells, as well as the hypertrophy of the renal tubule. Importantly, the data implicate that ICAM-1 can be used a biomarker for prediction of diabetes and DN, and may also be serviced as a target for drug development.[35] Results from experimental studies suggested that modulation of these cytokines may have significant clinical application as adjuvant therapy for DN. The present study showed a significant increase of plasma ICAM-1 and TNF-α levels in diabetic rats. Either rosiglitazone or pravastatin treatment partially reduced their levels. Additionally, a more significant decrease was found in rosiglitazone, or pravastatin treated groups. Similar results were reported by Rao et al.[36] Therefore, rosiglitazone or pravastatin may have beneficial effects by attenuating the inflammation-associated progression of DN.

Lipid peroxidation plays an important role in the development of complications of diabetes.[37] In the present study, diabetes has been noted to increase lipid peroxide level. Thus, it may be suggested that diabetes-induced development of oxidative stress may induce nephropathy by damaging renal architecture. The administration of either rosiglitazone, or pravastatin reduced plasma lipid peroxide levels. Statins have been proven to have antioxidant effects via elimination of free radicals directly, blockage synthesis of mediates important for post-translational modifications of proteins, or promotion synthesis of nuclear factor that protects low density proteins from oxidative stress.[38] Attenuation of the diabetes-induced increase in serum lipid peroxide observed in the present study by combined therapy with rosiglitazone and pravastatin proves that both of them have antioxidant properties that can improve DN. Taken together, the overall observed beneficial effect of a combination of rosiglitazone and pravastatin in attenuating the development of DN may be attributed to their direct renoprotective action, reduction in high circulating lipids and inflammatory markers, and prevention of oxidative stress. Promisingly, these initial experimental results suggest that a combination of rosiglitazone

and pravastatin could be beneficial in attenuating the development of DN. However, we aspire to be proven right, so far, combined rosiglitazone and pravastatin therapy will have dramatic effect on improving DN.

A limitation of this study was the relatively small sample size, and for this reason, this findings cannot be generalized on a broader community based on this study alone.

In conclusion, the combination strategy of rosiglitazone and pravastatin may provide potential synergistic renoprotective effect against DN by improving renal function and reducing indices of DN. Therefore, long-term prospective clinical studies investigating the combination strategy of rosiglitazone and pravastatin are important to clarify their clinical renoprotective effect without producing side effects.

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