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Differential Renoprotective Actions of Simvastatin and Rosuvastatin in Streptozotocin-Induced Diabetes in Sprague-Dawley Rat

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ABSTRACT

Diabetic nephropathy (DN) is the most frequent cause of the end-stage renal disease (ESRD) in about 33% of diabetic patients. The present study aimed to explore the renoprotective effects of simvastatin (SV) and rosuvastatin (RSU) on the kidney of streptozotocin (STZ)-induced diabetic Sprague-Dawley (SD) rat model. As a result of induction of diabetes, serum [cystatin C, transforming growth factor-beta (TGF-β), and 8-hydroxy-2' -deoxyguanosine (8-OHdG)] and tissue [interleukin 1 beta (IL-1β), interleukin 10 (IL-10), prostaglandin E2 (PGE2), cytochrome c, malondialdehyde (MDA), glutathione (GSH), glutathione disulfide (GSSG), GSH/GSSG ratio) markers showed significant increase than negative controls except tissue total glutathione (tGSH). Both SV and RSU significantly shifted the levels back toward near non-diabetic values. Both exerted the same renoprotective effect indicated by a significant decrease in cystatin C. However, SV significantly lowered IL-10, GSH/GSSG ratio, MDA, and 8-OHdG than RSU. Similarly, RSU significantly lowered cytochrome c and GSSG than SV. In conclusion, SV and RSU have differential renoprotective effects via alteration of growth factor, inflammatory, oxidative stress, DNA damage, and apoptotic signaling pathways.

Keywords: *Diabetic Nephropathy; Rat Model; Rosuvastatin; Simvastatin; Streptozotocin.*

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1. INTRODUCTION

The impact of diabetes on health resides in a series of complications that characterize this disease **[1]**. Hyperglycemia-induced microvascular complications are the main cause of diabetic nephropathy (DN). Approximately one-third of all diabetic patients will develop DN **[2, 3]**, which constitutes the most frequent cause of the end-stage renal disease (ESRD) [1]. Reactive Oxygen Species (ROS) and subsequent cellular damage trigger induction of glucose homeostasis transcriptional factors, the proinflammatory cytokines (such as IL-1β), and growth factors (such as TGF-β1) **[4-6]**. The mechanism by which oxidative stress can induce apoptosis may involve increased peroxidation of membrane lipids, increased oxidative injury to macromolecules, changes in cellular redox potential, or depletion of glutathione **[7, 8]**. ROS-

induced hyperglycemia also affects mitochondrial function and mitochondrial oxidative stress in turn increases apoptosis and leads to diminished beta-cell mass or loss of beta-cell function **[9]**.

Serum cystatin C, a non-glycosylated (13.3-kDa) cystatin protease inhibitor, is a promising replacement for serum creatinine in detecting the estimated glomerular filtration rate (eGFR) **[10]**. Murty *et al*., **[11]** showed that serum cystatin C is an ideal marker of renal function in the early stages of acute kidney injury (AKI). Unlike serum creatinine, it is less affected by age, gender, ethnicity, muscle mass, protein catabolism, or dietetic factors. Other studies showed that serum cystatin C level might be increased by diabetes, inflammation, and TGF-β (a marker of inflammation/endothelial damage/fibrosis) **[10, 12]**.

Statins are widely used to reduce cardiovascular risks in diabetic patients **[13-15]**. Some statins showed renoprotective actions **[16-18]**. Such actions are probably mediated by pleiotropic mechanisms including actions on cell proliferation, apoptosis, oxidative stress, and inflammation, which may augment the progress of DN **[19]**. Hussein & Mahfouz **[18]** showed that oral administration of resveratrol (RSV) with RSU improved glycemic control and attenuated oxidative stress damage in renal tissues of diabetic albino rats.

The solubility of statins is a crucial rate-limiting factor for their bioavailability and pharmacological response. Various enhancement solubility techniques such as inclusion complex formation, solid dispersion, solubilization by surfactants, and particle size reduction techniques have been successfully employed to improve the solubility of SV **[20]**. SV exerts cardioprotective effects via attenuation of hyperglycemia/hyperlipidemia-induced oxidative stress, enhanced antioxidant defenses, ameliorated cardiac hypertrophy, inflammation, apoptosis, fibrosis, and lowering serum tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) in STZ-induced diabetes in Wister rats **[15]**. Statins including RUS are proposed to alter molecular pathways of oxidation, inflammation, and apoptosis via their positive effect on low-density lipoprotein receptor (LDL-R) as well **[21]**.

The current study aimed to assess and compare the pleiotropic actions of the water-soluble RSU versus the lipid-soluble SV on cellular damage in diabetes-induced nephropathy in SD rats.

2. MATERIALS AND METHODS

Thirty-two adult males of SD rats (weighing 140-150 g each) were used. Animals were kept under observation for one week before the study with free access to feed and water. Rats were housed under controlled conditions of light illumination, relative humidity, and temperature of 22-25 °C. All animal procedures were performed according to an approved protocol and following regulations of the local committee on the care and use of experimental animals of Alexandria University according to the guidelines of the National Institutes of Health (NIH).

2.1. Drug treatments

Diabetes was induced by a single intraperitoneal injection of STZ [65 mg/kg; dissolved in citrate buffer (0.1 M, pH 4.5) **[22]** into 12 h food-deprived rats **[23]**. Induction of diabetes was confirmed by a test for blood glucose level >200 mg/dL (blood samples were withdrawn from the tail vein), 48 hours after STZ injection **[6]**. Statins were dissolved in 0.5% methylcellulose and treatment was started 3 weeks after STZ injection when the kidney should have recovered from the acute mild nephrotoxic effect of STZ **[24]**.

The 32 rats used in the study were divided into the following four groups (eight rats each):

Group I: Non-diabetic rats served as negative control and received 0.2 mL of the drug vehicle (0.5% methylcellulose) by oral gavage for 2 weeks.

Group II: Diabetic rats served as positive control and received 0.2 mL of the vehicle (0.5% methylcellulose), orally for 2 weeks.

Group III: Diabetic rats received SV (10 mg/kg/day in 0.5% methyl cellulose) orally for 2 weeks **[25]**.

Group IV: Diabetic rats received RSU (10 mg/kg/day in 0.5% methyl cellulose) orally for 2 weeks **[18]**.

2.2. Methods

At the end of each treatment period, animals were sacrificed by cervical dislocation. Blood was collected from the descending aorta through a laparotomy incision, serum was separated and used for determination of:

- Cystatin C as an estimator of glomerular filtration rate **[26, 27]**.
- TGF-β as one of the growth factors which are implicated in diabetic nephropathy pathogenesis **[26, 27]**.
- 8-OHdG as a marker for oxidative DNA damage **[28]**.

Kidneys were quickly excised, washed with ice-cold saline and immediately kept at -80 °C until homogenized and assayed for the following parameters:

- Cytokines; including the pro-inflammatory IL-1β and the anti-inflammatory IL-10 **[29]**.
- PGE2 **[30]**.
- MDA **[31]**, GSH, GSSG, GSH/GSSG ratio **[32]** and $_t$GSH calculated as (2 GSSG + GSH).
- Cytochrome c as an indicator of apoptosis **[33]**.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics for windows, version 20 (Armonk, NY: IBM Corp. 2011). Quantitative data were expressed in mean ± standard deviation (Mean ± SD). Differences between means were assessed by one-way analysis of variance (ANOVA) followed by Tukey's procedure and were considered statistically significant at $p< 0.05$.

3. Results

3.1. Kidney

Changes in inflammatory mediators and apoptotic markers in the kidney of diabetic rats following treatment with SV and RUS are presented in **Table 1**. All values for the determined parameters increased significantly by induction of diabetes and were attenuated by treatment with both statin compounds used ($p< 0.05$).

The mean level of IL-1β in diabetic rats was significantly elevated reaching about 3.4 times the negative control value. Treatment with the statins used in the present study caused a significant lowering of this marker reaching 38.1% and 26.9% below the diabetic untreated group for the SV and RSU treated groups; respectively. However, these values were still significantly higher than the normal controls.

The changes in the levels of IL-10 were qualitatively similar to those of IL-1β. The mean level in the diabetic kidneys was 6.6 times the normal control and decreased significantly by 63.4% after treatment with SV and by 47.7% with RSU. These levels were still significantly higher than the normal value and the mean level of the RSU group was significantly higher than after SV by 42.6%.

Table 1. Inflammatory and apoptotic markers in kidney tissue of STZ-induced diabetic rats following treatment with SV and RSU

Rat Groups	IL-1β (pg/g tissue)	IL-10 (pg/g tissue)	PGE2 (pg/g tissue)	Cytochrome c (pg/g tissue)
Normal group	31.95 ± 2.99	15.30 ±1.81	66.58±3.54	1.64± 0.27
Diabetic group	108.6 ± 13.02*	101.1± 5.19*	209.3±9.16*	8.87±0.77*
Simvastatin-treated diabetic group	67.45± 5.92$^{*\#}$	37.05±3.78$^{*\#}$	94.55±7.97$^{*\#}$	3.13±0.34$^{*\#}$
Rosuvastatin-treated diabetic group	79.35 ± 6.25$^{*\#}$	52.9±8.15$^{*\#@}$	97.68±8.06$^{*\#}$	2.17±0.36$^{\#@}$

***, Significant difference between normal (negative control) and each other group; #, the significant difference between diabetic (positive control) and simvastatin or rosuvastatin; @, the significant difference between simvastatin and rosuvastatin; IL-1β, Interleukin-1β; IL-10, Interleukin-10; PGE2, Prostaglandin E2; 6 rats in each group.**

Table 2. Changes in oxidative stress markers in the kidney tissues of streptozotocin-induced diabetic rats following treatment with simvastatin and rosuvastatin

Rat Groups	GSH (μmol/L)	GSSG (μmol/L)	tGSH (μmol/L)	GSH/GSSG Ratio	MDA (nmol/L)
Normal group	47.23±2.57	6.49±0.38	60.2±2.58	7.30 ±0.60	11.47±1.57
Diabetic group	18.13±2.06*	21.33±2.63*	62.0±5.83	0.84±0.14*	66.65±4.61*
Simvastatin-treated diabetic group	40.2±4.55$^{*\#}$	10.73 ±0.75$^{*\#}$	61.67±5.56	3.75±0.34$^{*\#}$	27.43±3.38$^{*\#}$
Rosuvastatin-treated diabetic group	43.87±3.16$^{\#}$	7.31 ±1$^{\#@}$	58.48±4.79	6.07±0.6$^{*\#@}$	36.32±2.88$^{*\#@}$

GSH, reduced glutathione; GSSG, oxidized glutathione; tGSH, total glutathione (= 2GSSG + GSH); MDA, malondialdehyde. *, significant difference between normal (negative control) & each other group; #, significant difference between diabetic (positive control) & simvastatin or rosuvastatin; @, significant difference between simvastatin & rosuvastatin; 6 rats in each group.

Induction of diabetes caused an elevation in PGE2 reaching values ~3.1 times control. Although treatment with SV decreased PGE2 level by 54.8% and by 53.3% after RSU, these levels were significantly higher than the negative control.

The level of cytochrome c in diabetic kidney was 5.4 times that of the negative control. Treatment with the statins used caused significant decreases of 64.9% with SV and 75.5% with RSU. However, there was a statistical difference between the levels of the two statins treated groups, as rosuvastatin gave a higher effect.

The effects of induction of diabetes and treatment with SV and RSU on oxidative stress markers are presented in **Table 2**. GSH decreased by 61.6% in the diabetic group and returned to near normal by treatment with either statin used. The level of GSSG in diabetics was almost 3.3 times the normal level and decreased by 49.7% after treatment with SV and by 65.7% with RSU. No difference was detected in total glutathione in all tested groups. Increased oxidative stress was clear from the GSH/GSSG ratio, which decreased by 88.5% below normal as a result of induction of diabetes but went back to near normal reaching 4.46 times the diabetic level in the SV group and 7.22 times in the RSU group. The value in the RSU group was significantly higher than in the SV group by 61.8%.

The lipid peroxidation products, estimated as

MDA, were significantly elevated to reach concentrations 5.8 times the normal control. In both drug-treated groups, renal concentrations were lower by 58.8% and 45.5% below the diabetic level in the SV and RSU groups; respectively.

3.2. Serum

Data on the serum markers of kidney damage are presented in **Table 3**. The mean level of cystatin C in the sera of diabetic rats was 42.2 times that of normal controls. Treatment with SV decreased cystatin C by 68.6% while treatment with RSU decreased it by 74.2%, below positive control, however, values for both treatments were still significantly higher than normal control.

Oxidative damage of nucleic acids, represented by 8-OHdG, as a result of diabetes induction, was evident. The level of this marker increased in the diabetic sera to 9.77 times that of the normal rats. Treatment with SV decreases 8-OHdG level by 74.7% below that of the diabetic untreated group while treatment with RSU decreased it by 66.2%. The level in the RSU group was significantly higher than the SV group by 33.7%.

Table 3. Serum markers of kidney damage in streptozotocin-induced diabetic rats following treatment with simvastatin and rosuvastatin

Rat Groups	Cystatin C (ng/mL)	TGF-β1 (pg/mL)	8-OHdG (ng/mL)
Normal group	0.19± 0.03	20.28± 1.78	1.02± 0.12
Diabetic group	8.02 ± 0.80*	119.92±4.76*	9.97±0.78*
Simvastatin-treated diabetic group	2.52 ± 0.25$^{*\#}$	45.82±2.78$^{*\#}$	2.52 ± 0.31$^{*\#}$
Rosuvastatin-treated diabetic group	2.07±0.34$^{*\#}$	39.33± 6.26$^{*\#}$	3.37± 0.33$^{*\#@}$

***, significant difference between normal (negative control) & each other group; #, significant difference between diabetic (positive control) & simvastatin or rosuvastatin; @, significant difference between simvastatin & rosuvastatin; TGF-β1, transforming growth factor- β1; 8-OHdG, 8-hydroxydeoxyguanosine; 6 rats in each group.**

4. Discussion

The pathogenesis of DN appears to be multifactorial with dyslipidemia as a co-morbidity, which may influence the development and progression of damage in the diabetic kidney **[34, 35]**. The renoprotective action of statins in diabetes comes either from the hypolipidemic effect, which is associated with decreased albuminuria and diabetic kidney disease **[36]** or lipid-independent actions on processes which may augment the progress of DN. Pleiotropic mechanisms of statins, including actions on cell proliferation/apoptosis and oxidative stress, may exert beneficial effects independent of their lipid-modifying properties **[19]**, as presented in the results of the present study. Disturbances in the levels of inflammatory, apoptotic, and oxidative stress markers were observed as a result of the induction of diabetes, which was partly corrected by treatment with the used statins. The mean increase level of IL-1β, which is an important mediator of the inflammatory response in diabetic rats, was several times that of normal controls. These high levels were attenuated approximately to the same levels by treatment with either SV or RUS.

It is noteworthy that the increase in the proinflammatory IL-1β was accompanied by a significant increase in IL-10, which is a potent anti-inflammatory agent by its ability to suppress genes for proinflammatory cytokines **[37]**. Such probably represented a defense mechanism

against the high levels of the proinflammatory mediators in the diabetic kidney. It has been proposed that the biological activities of IL-10 in modulating inflammation may be caused, in part, by downregulation of proinflammatory cytokines and their receptors and upregulation of cytokine inhibitors **[38]**. Treatment with the statins caused a significant decrease in IL-10 parallel to the decrease in IL-1β, with RSU more effective than SV.

Induction of diabetes caused PGE2 to reach higher levels in the kidneys of the diabetic rats, which were partially corrected by treatment with statins. An increase in the local production of prostaglandins in the kidney has been observed in clinical and experimental DN and prostaglandin synthesis is augmented in glomeruli of STZ-induced diabetic rats **[39]**. The overproduction of PGE2 plays an important role in the end-organ damage in diabetes **[40]**. It was suggested that IL-1 preferentially stimulates the production of prostaglandins and many of the biological activities of IL-1 are probably due to increased PGE2 production **[39]**. The decrease of IL-1 level following treatment with both statins used was accompanied by a similar fall in PGE2. This may be a pointer for possible attenuation of the progression of kidney damage.

It was also suggested that increased oxidative stress and increased levels of inflammatory cytokines, like TGF-β, may enhance the apoptosis levels in DN **[41]**. TGF β-1 increases intracellular ROS in mesangial and tubular epithelial cells **[42]**. Oxidative stress is clearly shown in the diabetic kidney in the present study by decreased levels of GSH and GSH/GSSG ratio. On the other hand, there are substantial increases in the levels of GSSG and MDA. These changes were shifted toward the non-diabetic values following treatment with both statins used. However, the level of total glutathione was not modified either by induction of diabetes or by treatment with statins. This may indicate that the synthesis of glutathione was not affected by these manipulations and the problem lies with the reduction of GSSG.

Fragmentations of nucleic acids, as shown by the serum level of 8-hydroxyguanosine and the apoptotic marker cytochrome c were highly elevated in diabetic rats. Apoptosis contributes to the development of diabetic nephropathy. It was suggested that increased oxidative stress and increased levels of inflammatory cytokines may also enhance the apoptosis levels in DN **[41]**. When the cell detects an apoptotic stimulus, such as DNA damage or metabolic stress, the intrinsic apoptotic pathway is triggered, and mitochondrial cytochrome c is released into the cytosol **[43]**. It has been suggested that cystatin C might predict the risk of developing chronic kidney disease thereby signaling a state of preclinical kidney dysfunction **[44]**. Attenuation of the high levels of these parameters is therefore expected to improve renal function and to slow the progression of kidney disease.

Conclusion

Treatment of diabetic rats with simvastatin or rosuvastatin caused the determined inflammatory, oxidative stress, and apoptosis markers to shift toward near normal values and therefore probably slowing down the progression of renal dysfunction in this model.

Declarations

Ethical approval

Ethics committee approval was stated clearly in the materials and methods section.

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the

main manuscript.

Competing interests

The authors declare no competing interests.

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Authors' contributions

All authors have contributed equally to this work.

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List of abbreviations

8-OHdG, 8-hydroxy-2' –deoxyguanosine; AKI, acute kidney injury; CRP, C-reactive protein; DN, Diabetic nephropathy; eGFR, estimated glomerular filtration rate; ESRD, End-stage renal disease; GSH, Glutathione; GSSG, glutathione disulfide; IL-10, Interleukin 10; IL-1β, Interleukin 1 beta; LDL-R, Low-density lipoprotein receptor; MDA, Malondialdehyde; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NIH, National Institutes of Health; PGE2, Prostaglandin E2; ROS, Reactive Oxygen Species; RSU, Rosuvastatin; RSV, Resveratrol; SD, Sprague-Dawley, SV, Simvastatin; TGF-β, Transforming growth factor-beta; tGSH, Total glutathione; TNF-α, Tumor necrosis factor-α; STZ, Streptozotocin.

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