Sitagliptin Mitigates Diabetic Nephropathy in a Rat Model of Streptozotocin-Induced Type 2 Diabetes: Possible Role of PTP1B/JAK-STAT Pathway

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INTRODUCTION

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus that commonly leads to end-stage renal disease. The pathophysiology of diabetic-induced nephropathy is complex, and its precise mechanism is not fully understood. When the hormones of the incretins group become dysregulated, namely, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), they contribute to DN development. Dipeptidyl peptidase-4 (DPP-4) deactivates GIP and GLP-1, and therefore DPP-4 inhibitors have a positive impact on microvascular complications and improve the outcomes of DN (Avogaro et al., 2014).

Protein tyrosine phosphatase 1B (PTP1B) plays a critical role in controlling glucose uptake and regulating insulin. It can also be considered a negative regulator of the JAK-STAT signaling pathway, one that results in the alleviation of inflammation (Marrero et al., 2006). Therefore, there is a key link between PTP1B and inflammation, which plays a major role in DN development.

Unfortunately, current data on the relationship between DPP-4 inhibition and the PTP1B/JAK-STAT pathway and its effect on DN is insufficient to drawing robust conclusions, however, there is data suggesting that sitagliptin, a DPP-4 inhibitor, can mitigate oxidative stress and inflammation (Wang et al., 2018), which plays a major role in DN pathogenesis.

RESEARCH HYPOTHESIS

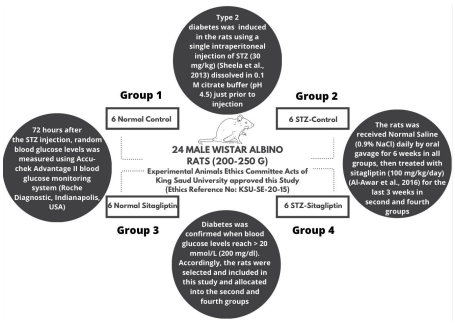
Due to data indicating that sitagliptin, a DDP-4 inhibitor, can mitigate oxidative stress and inflammation, we hypothesized that sitagliptin may attenuate DN through the modulation of PTP1B and the JAK-STAT pathway.

OBJECTIVES

- To elucidate the renoprotective effect mediated by sitagliptin by assessing kidney injury and inflammatory biomarkers in streptozotocin (STZ)-induced DN in rats.
- To investigate the mechanism underlying attenuation of renal injury mediated by sitagliptin via detection of PTP1B and p-JAK2/p-STAT3 protein levels in the kidneys of type 2 diabetic rats.

METHODS

Experimental design:



Biochemical and molecular analysis:

Serum Glucose Levels	It was measured using a Glucose analytical assay kit according to the manufacturer`s instructions.
Diabetic Nephropathy Biomarkers	Kidney biomarkers (albumin, creatinine, and urea) were determined using serum samples and rat ELISA kits from Abcam (Biotechnology Inc., UK).
Inflammatory Biomarkers	Serum inflammatory cytokines IL-6 and TNF-α levels were measured using ELISA kits, following the manufacturer's instructions (Abcam, Biotechnology, Inc, UK).
Western Blot Analysis	Western blot analysis was performed as described previously by Towbin et al. (1979) to detect expression levels of the targeted proteins (PTP1B and P-JAK2/P-STAT3) in kidney tissue homogenates.
Histological Examination	The rat's kidneys were embedded within paraffin, then prepared sections (4-µm) were stained with hematoxylin and eosin (H&E). It was incubated overnight with antibodies and analyzed the images by specialized investigators.

Statistical analysis:

Data are expressed as means ± SEM. Statistical analysis was conducted using Prism Windows software Version 5 (Graphpad Software Inc., San Diego, USA). One-way analysis of variance (ANOVA) followed by Tukey-Kramer post-hoc test. P values < 0.05 will be considered statistically significant.

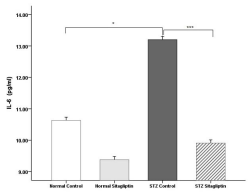
RESULTS

Table 1. Effects of sitagliptin on glucose, kidney weight, kidney/body weight ratio (%) and DN biomarkers in STZ-induced diabetic rats

	Glucose (mg/dl)	Kidney Weight (g)	Kidney/Body Weight Ratio (%) (mg/g)	Urea (mg/dl)	BUN (mg/dl)	Creatinine (IU/L)
Normal-Control	3.72 ± 0.58	1.83 ± 0.16 ###	0.57 ± 0.05	11.92 ± 2.85 ###	5.56 ± 1.33	0.28 ± 0.03
Normal-Sitagliptin	3.98 ± 0.25 ###	2.00 ± 0.06 ##	0.74 ± 0.03	9.65 ± 1.13 ###	4.50 ± 0.53 ###	0.25 ± 0.44 ###
STZ-Control	9.21 ± 1.41 ###	2.80 ± 0.25	1.04 ± 0.08 ###	49.70 ± 4.02	23.19 ± 1.87 ###	0.64 ± 0.10 ##
STZ-Sitagliptin	4.26 ± 1.06 ###	1.87 ± 0.05 ###	0.68 ± 0.02 ###	35.31 ± 10.67 *	16.47 ± 4.98 *	0.31 ± 0.08 *

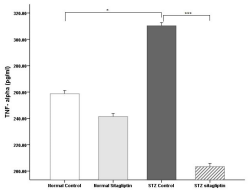
Data are expressed as means ± SEM (N= 8 samples/group). *P < 0.05, **P < 0.01, ***P < 0.001 compared to STZ-Control and non-diabetic control group.

Figure 1. The Effects of sitagliptin on interleukin-6 (IL-6) expression in STZ-induced diabetic rats



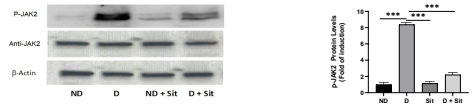
*P < 0.05, **P < 0.01, ***P < 0.001.

Figure 2. The Effects of sitagliptin on tumour necrosis factor alpha (TNF-α) expression in STZ-induced diabetic rats



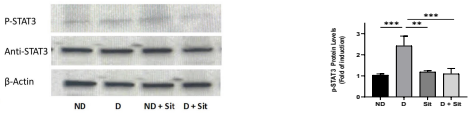
*P < 0.05, **P < 0.01, ***P < 0.001.

Figure 3. The effect of sitagliptin on P-JAK2 expressions in STZ-induced diabetic rats



(ND) Normal control, (D) STZ control, (ND+Sit) Normal sitagliptin, (D+Sit) STZ Sitagliptin, *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 4. The effect of sitagliptin on P-STAT3 expressions in STZ-induced diabetic rats

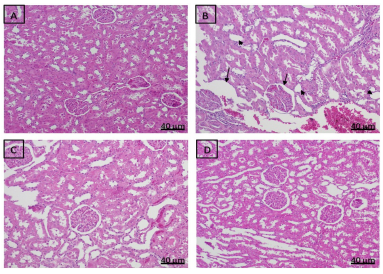


(ND) Normal control, (D) STZ control, (ND+Sit) Normal sitagliptin, (D+Sit) STZ Sitagliptin, *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 5. The effect of sitagliptin on PTP1B expressions in STZ-induced diabetic rats.

(ND) Normal control, (D) STZ control, (ND+Sit) Normal sitagliptin, (D+Sit) STZ Sitagliptin, *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 6. Histopathological changes in experimental diabetic rats kidneys treated with sitagliptin



Photomicrograph of kidney sections stained with hematoxylin and eosin (H&E) showing: (A) normal control rats; (B) diabetic STZ control rats; (C) Sitagliptin normal rats (100 mg/kg/day, p.o.); and (D) diabetic STZ rats treated with sitagliptin.

CONCLUSION

The results of this study suggest that sitagliptin attenuated DN via the modulation of PTP1B and JAK/STAT signaling pathway. Thus, sitagliptin may be considered a useful therapeutic agent in the treatment of DN.

REFERENCES