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The effects of a sodium-glucose cotransporter 2 inhibitor on diabetic nephropathy and serum oxidized low-density lipoprotein levels

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Abstract. – OBJECTIVE: To investigate the effect of an SGLT-2 inhibitor on diabetic nephropathy and serum oxidized low-density lipoprotein (ox-LDL) levels.

PATIENTS AND METHODS: We randomly divided 126 patients with diabetic nephropathy into the treatment group and control group. The 63 patients in the treatment group received an SGLT-2 inhibitor in addition to routine insulin therapy, while the control group received only insulin to control blood glucose. All laboratory indexes were recorded before and after treatment with the SGLT-2 inhibitor. The prognosis of patients was followed-up. Simultaneously, 63 healthy and BMI-matched in-patients were selected as the healthy control group. Peripheral blood samples were collected from all groups, and the levels of ROS were measured by ELISA.

RESULTS: Renal function indexes such as urinary protein, creatinine, blood urea nitrogen, and glomerular filtration rate (GFR) were significantly higher with SGLT-2 inhibitor treatment compared with the control group ($p<0.05$). The fasting blood glucose level was not significantly increased before or after treatment ($p>0.05$). The levels of ROS in peripheral blood were significantly lower in the treatment group than in the control group ($p<0.05$). Observation at the 1-year follow-up showed that the average GFR was significantly higher in the treatment group than in the control group. Furthermore, the proportion of patients with stage 1-3 chronic kidney disease was significantly higher in the treatment group than in the control group ($p<0.05$).

CONCLUSIONS: The SGLT-2 inhibitor had a good therapeutic effect on renal function in patients with diabetic nephropathy, without having effects on fasting blood glucose. Additionally, it significantly delayed the progression of nephropathy. It is therefore worth clinical promotion.

Key Words

Sodium-glucose cotransporter 2 (SGLT-2), Diabetic nephropathy, Blood glucose.

Introduction

In recent years, our country has made great progress in healthcare. However, diabetes and its complications remain major threats to human health[1]. Studies have shown that diabetes-induced abnormalities of lipid metabolism may play a role in the development of diabetic nephropathy[2]. In addition, recent research shows that oxidized low-density lipoprotein (ox-LDL) may accelerate the injury of glomerular mesangial cells and podocytes, and increase both glomerular filtration rate (GFR) and proteinuria[3]. Therefore, it may represent an important cause of diabetic nephropathy. Previous studies indicated that oxidative stress is closely related to the development and progression of inflammatory and vascular diseases[4-6], and associated metabolic syndromes, including insulin resistance, obesity, hypertension, and dyslipidemia[7-8]. Plasma ox-LDL is generally considered to be a common marker of oxidative stress. A previous study[9] showed that, compared with patients with non-metabolic syndrome, ox-LDL levels in patients with metabolic syndrome were significantly increased. Some reports showed that ox-LDL, rather than LDL, might have value for predicting insulin-treated Type 2 Diabetes (T2D). This suggests that oxidative modifications of LDL can be used as indicators of metabolic changes[10]. By measuring serum reactive oxygen species (ROS), it is possible to identify metabolic changes of ox-LDL. These changes may occur in both patients suffering from T2D and subjects who will develop T2D[11].

Dapagliflozin, the second Food and Drug Administration (FDA)-approved Sodium-Glucose Cotransporter 2 (SGLT-2) inhibitor, acts primarily by inhibiting renal glucose reabsorption to

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increase urinary glucose excretion and improve hyperglycemia in diabetic patients. This mechanism is uncomplicated and has no effect on insulin-related metabolic changes.

A recent study showed that dapagliflozin has an excellent effect on improving ox-LDL accumulation induced by type 2 diabetes and/or hyperlipidemia, especially for lipid accumulation and foam cell formation on vascular endothelium, whose effect is related to inhibiting activation of the AKT signaling pathway.

However, the effect of dapagliflozin in ox-LDL-injured renal tubular epithelial cells has not been fully investigated. Based on previous research, the aim of the present study was to examine the role of ox-LDL in renal tubular epithelial cell injury. In addition, we examined the renal protective effect of the STLG-2 inhibitor on an ox-LDL-induced renal tubular epithelial cell injury model. This study also further clarified the mechanism related to the occurrence and development of diabetic nephropathy, and protection of drug treatment.

Although the relationship between ox-LDL and diabetes has been well studied, the results are inconsistent and inconclusive. In this study, patients with diabetic nephropathy were treated with SGLT-2 inhibitors and followed-up for 1 year to observe the therapeutic effect.

Patients and Methods

Inclusion Criteria

(1) Diabetic nephropathy was confirmed in patients by laboratory examination, pathological examination, and clinical examination; (2) age ≥ 18 years old.

Exclusion Criteria

Patients with (1) urinary tumors; (2) myocardial infarction, aortic aneurysm, aortic dissection, cerebral hemorrhage, stroke, pheochromocytoma, thyroid dysfunction, or any other renal disease that may affect arterial blood pressure or lead to secondary hypertension; (3) infection caused by surgery, accident, trauma, or other factors; (4) cognitive dysfunction or mental disorder; (5) recent history of hypovolemic shock or blood flow decline caused by other factors, blood transfusion; (6) alcoholic liver disease, drug-induced liver disease, autoimmune liver disease; (7) coagulation dysfunction; (8) allergy to SGLT-2 inhibitors; (9) poor compliance.

Clinical Information

This was a prospective study. We enrolled 126 patients with chronic kidney disease, including 55 males and 71 females aged from 31-68 years old (mean: 58.7 ± 2.3 years), with an 8-month to 7.5-year (median 4.5 years) course of the disease. All patients were evaluated according to a standard protocol for evaluation of chronic diabetic nephropathy. This included laboratory examination, pathological examination, and clinical examination. This investigation was approved by our Hospital Ethics Committee. Also, all the patients signed the informed consent.

Methods

Patients with diabetic nephropathy received 0.05 g/day Dapagliflozin (Squibb) for 6 months.

Classification of Chronic Kidney Disease

According to the classifications recommended by the Kidney Disease Quality Outcome Initiative (K/DOQI)[7], stage 1 CKD is defined as kidney damage or GFR ≥90 mL/min/1.73 m^2, as shown in Table I.

Table I. Classification of chronic kidney disease (CKD).

Stage	Description	GFR [ml/(min • 1.73 m^2)]	Action
1	Kidney damage with normal or ↑ GFR	≥90	Normal GFR, Treatment for nephrotic syndrome
2	Kidney damage with mild ↓ GFR	60-89	Slowing progression and reducing CVD risk
3	Moderate ↓ GFR	30-59	Slowing progression, Evaluating and treating complications
4	Severe ↓ GFR	15-29	Comprehensive treatment, treating complications
5	Kidney failure	<15	Preparation for dialysis (or dialysis)

Table II. Baseline parameters of patients and controls (X±s).

Group	Cases (n)	Age (years)	BMI (kg/m^2)	MAP (mmHg)
Treatment	126	44.5±12.7	20.7±1.2	118.3±12.4
Control	126	51.6±10.8	21.2±1.3	76.5±10.9
t-value	-	0.33	1.29	21.43
p-value	-	0.47	0.22	0.02

ELISA

(1) Plasma pretreatment: Within 30 min after blood was collected, EDTA anticoagulants were added. Samples were centrifuged at 2000 rpm and 2-8°C for 30 min.
(2) The strips required for experiments were equilibrated at room temperature for 20 min and removed from the aluminum foil bag.
(3) A total of 50 µL of prepared standards and samples were added to wells. A blank well was used as the control.
(4) A total of 100 µL HRP conjugate was added to each well, except for control wells. Plates were covered and incubated at 37°C for 60 min.
(5) The solution from wells was thoroughly aspirated or decanted and discarded. A total of 350 µL washing solution was added for 1 min to wash wells. The wash was repeated 4-5 times.
(6) A total of 50 µL of solution A and 50 µL of solution B were added to each well and maintained at 37°C in the dark for 15 min.
(7) A total of 50 µL of stop solution was added to each well, and plates were evaluated within 15 min. The absorbance (OD) was read at 450 nm.
(8) Control values were subtracted from standard values. Curve-fitting statistical software was used to plot a four-parameter logistic curve fit to the standards. The results of the test samples were then calculated.

Statistical Analysis

SPSS19.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for data analysis. Quantitative data were analyzed by one-way analysis of variance (ANOVA); qualitative data were analyzed by χ^2-test. In cases where data did not meet the required conditions, the Fisher test was conducted to calculate the exact probability. Comparisons were considered statistically significant at $p<0.05$.

Results

Comparison of Baseline Parameters Between Patients and Healthy Controls

Baseline parameters including age, mean arterial pressure (MAP), and BMI were recorded and statistically analyzed. There were no significant differences between the healthy control group and treatment group ($p>0.05$, Table II).

Comparisons of Glomerular Filtration Rate and Fasting Blood Glucose Before and After Treatment in Both Groups

Table III shows that GFR was significantly improved in the group treated with the SGLT-2 inhibitor compared with the control group ($p<0.05$). There was no significant difference in fasting blood glucose (FBG) between the two groups ($p> 0.05$).

Table III. Comparisons of filtration rate (GFR) and fasting blood glucose (FBG) before and after treatment in both patient groups.

	Group	Case	Before treatment	After treatment	*t*-value	*p*-value
GFR (mL/min/1.73 m^2)	Treatment	63	45.3±12.1	76.4±21.2	32.42	0.006
	Control	63	42.3±10.3	43.3±10.9	0.28	0.41
	t-value	–	0.38	23.28	–	–
	p-value	–	0.42	0.001	–	–
Fasting blood glucose (mmol/L)	Treatment	63	8.81±2.33	7.69±0.42	0.43	0.74
	Control	63	8.32±1.42	7.83±0.82	0.51	0.42
	t-value	–	0.52	31.29	–	–
	p-value	–	0.43	0.002	–	–

Table IV. Baseline parameters of patients and controls (X±s).

Group	Case	Before treatment	After treatment	*t*-value	*p*-value
Treatment	63	345.3±29.1	76.4±21.2	32.42	0.006
Control	63	362.3±20.3	343.3±23.9	0.28	0.41
Health control	63	12.5±2.7	12.5±2.7	–	–
t-value	–	0.38	23.28	–	–
p-value	–	0.42	0.001	–	–

Measurement of ROS Levels in Each Group

Compared with the control group, treatment with the SGLT-2 inhibitor significantly reduced the levels of plasma ROS ($p<0.05$). While the levels of ROS were significantly lower in the treatment group than in the control group, they were higher than in the healthy controls ($p<0.05$) (Figure 1).

Follow-Up Results of The Two Patient Groups

During the follow-up period of 1 year, we found that the GFR levels of patients treated with the SGLT-2 inhibitor were significantly higher than those of the control group ($p<0.05$) (Table V and Figure 2).

Discussion

Dapagliflozin is a SGLT-2 inhibitor. On January 8 2014, the US FDA announced the approval of dapagliflozin for the treatment of T2D, based on the results of 16 clinical trials including 9,400 patients with T2D. These trials indicated that dapagliflozin can improve the levels of glycosylated hemoglobin A1c. Some studies[9-10] indicated that ROS are highly toxic to renal tubular epithelial cells and renal tubular endothelial cells. Also, they resulted in an enlargement of intercellular space, which may significantly affect the renal filtration function. This observation was confirmed in this study. We observed that treatment with the SGLT-2 inhibitor significantly reduced serum ROS levels and effectively improved renal function when compared with the control group. This shows that inhibiting SGLT-2 has a good anti-ROS effect that can improve the aggregation of ROS in patients with diabetic nephropathy, and reduce the damage to cells caused by peroxides produced by metabolic intermediates of oxygen.

In this study, by measuring urinary protein, creatinine, blood urea nitrogen, and GFR, we found that SGLT-2 inhibitor treatment significantly improved renal function when compared with the control group ($p<0.05$). However, FBG levels were not significantly increased ($p>0.05$) with SGLT-2 inhibitor treatment. As a result of a long-term disorder of glucose metabolism,

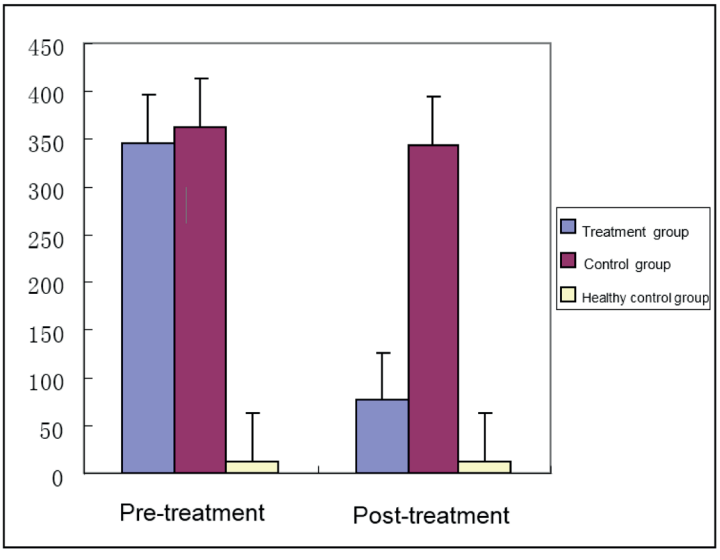

Figure 1. The levels of ROS in the three groups. Compared with the control group, treatment with the SGLT-2 inhibitor significantly reduced plasma ROS levels ($p<0.05$). The levels of ROS were significantly lower in the SGLT-2 inhibitor treated group compared with the control group, but higher than in the healthy control group ($p<0.05$).

Table V. One year follow-up results.

Group	Cases (n)	3-month	6-month	12-month
Treatment	63	52.3±12.2	55.3±18.5	64.4±12.5
control	63	51.2±9.8	45.3±9.4	54.3±10.7
t-value	-	0.28	0.42	30.38
p-value	-	0.77	0.52	0.008

disorders of aerobic metabolism in patients with diabetic nephropathy stimulate the production of large amounts of ROS[4]. These inflammatory factors can reach the kidneys via blood circulation, accumulate in glomerular and/or renal interstitium, and cause dysfunction of glomerular filtration and reabsorption. This may represent an etiology of diabetic nephropathy.

Although numerous studies on the relationship between ox-LDL and diabetes mellitus have been conducted, the results are neither consistent nor conclusive[12-13]. One reason is that diabetes is a systemic metabolic disease that is often accompanied by multiple organ damages, such as kidney damage and arterial endothelial injury. Some studies[14-18] have shown that ox-LDL, as a key mediator of cellular injury, acts via the following mechanisms: (1) ox-LDL promotes the secretion of adhesion molecules from endothelial cells and monocytes, and accelerates monocyte adhesion; (2) ox-LDL can inhibit the activity of superoxide dismutase and increase the production of oxygen free radicals. Under the condition of large amounts of ox-LDL, matrix malondialdehyde, an intermediate product of lipid peroxidation, increases intracellularly and extracellularly, and promotes ROS formation and membrane damage. Destruction of the membrane potential ultimately results in abnormal metabolism in healthy cells; (3) the dynamic balance between nitric oxide and endothelin determines a series of physiological processes including vascular relaxation, smooth muscle cell proliferation, and cell cycle progression in endothelial cells.

The role of ox-LDL in diabetic nephropathy has not been fully investigated. It is generally accepted that metabolic abnormalities of three major nutrients will create a pro-inflammatory microenvironment, leading to increased cellular ox-LDL-induced oxidative damage. Furthermore, hyperglycemia can promote oxidative stress in cell damage.

Studies have shown that over 40% of cases of T2D are associated with varying degrees of diabetic nephropathy, which eventually progresses to end-stage renal disease[19]. The end-stage renal disease is, in turn, the cause of death in some patients with T2D. Therefore, research on the pathogenesis of ox-LDL in renal tubular epithelial cells not only has great clinical significance for the prevention and treatment of diabetic nephropathy, but also has value for the development of targeted therapies.

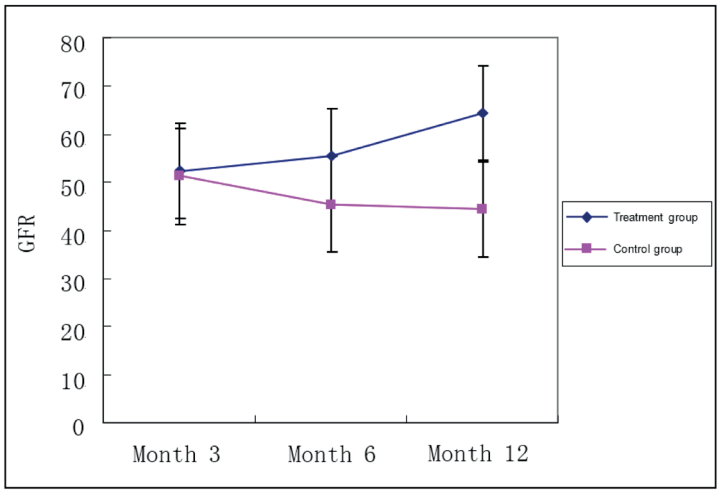

Figure 2. Changes of GFR in the two groups during the 1-year follow-up period. Compared with the control group, the GFR levels in the treatment group were significantly increased ($p<0.05$).

Conclusions

We demonstrated the efficacy of improving renal function with an SGLT-2 inhibitor in patients with diabetic nephropathy, without affecting FBG levels. Also, the SGLT-2 inhibitor significantly delayed the progression of nephropathy. These results suggest that SGLT-2 inhibitors should be popularized for clinical use.

Conflict of interest

The Authors declare that they have no conflict of interests.

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