

Dapagliflozin can alleviate renal fibrosis in rats with streptozotocin-induced type 2 diabetes mellitus

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Abstract. The aim of the present study was to explore the effects of Dapagliflozin on renal fibrosis in streptozotocin (STZ)-induced type 2 diabetes mellitus (T2DM) rats, and to determine the underlying mechanism of action. A total of 24 SPF male SD rats were randomly divided into 4 groups: A normal (Control) group, model group (STZ-induced T2DM rats), Dapagliflozin group (STZ-induced T2DM rats treated with 1 mg/kg Dapagliflozin), and a metformin group (STZ-induced T2DM rats treated with 200 mg/kg metformin), with 6 rats per a group. Peripheral blood and renal tissues were collected from these rats, and the renal indices of each group were examined. The fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), blood urea nitrogen (BUN), and serum creatinine (SCr) of rats were detected. After 24 h, the urine was collected and the urine protein levels were measured. Hematoxylin and eosin staining was used to detect histological changes in the rat kidney; Masson staining was used to observe the degree of fibrosis in rat renal tissues; and western blot was performed to determine the expression levels of α -smooth muscle actin (SMA), vimentin, E-cadherin, TGF- β 1, Smad7, and p-Smad3 in rat renal tissues. Dapagliflozin effectively inhibited the increase in FBG and HbA1c levels in diabetic mice, reduced renal tissue damage, reduced the renal index values, reduced collagen deposition in the glomerulus and interstitial area, and reduced the proliferation of glomerular mesangial cells. In addition, Dapagliflozin significantly lowered the levels of BUN, SCr, and 24-h urine protein, decreased the protein expression of α -SMA, vimentin, TGF- β 1, and p-Smad3, and increased the protein expression

levels of E-cadherin and Smad7. Together, these results showed that Dapagliflozin alleviated renal fibrosis in STZ-induced T2DM rats, and its mechanism of action may be related to the inhibition of the TGF- β 1/Smad pathway.

Introduction

Type 2 diabetes mellitus (T2DM) is a severe public health issue, and its prevalence is gradually increasing (1). Reports indicate that 629 million individuals will have T2DM by 2045, up from the current predicted 425 million sufferers (2). As T2DM and its complications are associated with very high rates of disability and mortality (3), researchers are committed to exploring safe and effective therapeutics to prevent and treat it. Diabetic nephropathy (DN, also known as diabetic kidney disease) is one of the most common chronic and destructive complications of diabetes. Approximately 20-40% of T2DM patients will develop DN (4), and this complication is most prevalent in developed countries (5). The pathological features of DN include proteinuria, glomerular hypertrophy, basement membrane thickening, podocytopenia, extracellular matrix protein deposition, and renal fibrosis. In particular, renal fibrosis is the primary pathological manifestation of renal injury (6,7). It is hypothesized that DN is the primary cause of death in diabetes patients as it can eventually progress to chronic renal failure (8). Therefore, timely and effective treatment for DN is of significant importance.

Currently, treatment goals for DN primarily include blood glucose control, reduction of hypertension, lipid control, and inhibition of renal fibrosis (9). First-line angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are drugs commonly used in clinical practice (10). However, the discontinuation of ACEIs and ARBs in patients with advanced chronic kidney disease (CKD) has been associated with a slowing or decreased estimated glomerular filtration rate (eGFR) (11). Therefore, alternative drugs have been successively developed in clinical practice, such as empagliflozin [a sodium-glucose cotransporter 2 (SGLT2) inhibitor], and fasudil [a Rho-associated coiled-coil-containing protein kinase (ROCK) inhibitor]. However, they also have significant side effects. For example, empagliflozin is unable to control blood glucose by regulating insulin (12), and although Fasudil can protect the kidneys from

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DN, it does not lower blood pressure (13). Poursharif *et al* (14) discovered that SGLT2 inhibitors improved renal function by slowing eGFR in patients with early and advanced DN. Several studies have shown that Dapagliflozin (a SGLT2 inhibitor) is a novel class of diabetes drugs and a clinically recognized therapeutic agent for DN (15-17). It can effectively reduce renal injury caused by DN and inhibit renal fibrosis. According to the Dapagliflozin and Prevention of Adverse Outcomes in CKD (DAPA-CKD) trial, Dapagliflozin lowered the risk of hospitalization for any reason (including heart disease, renal and urinary disorders, metabolic and nutritional disorders, and neoplasms) in CKD patients with or without type 2 diabetes (18,19). The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) trial results showed that Dapagliflozin significantly reduced the risk of cardiovascular (CV)-associated death in adults with T2DM and established CV disease compared with placebo (20). Thus, Dapagliflozin has a promising treatment option for DN. However, the mechanism of action of Dapagliflozin in the treatment of DN is still not fully elucidated, and additional scientific investigation is needed to improve our understanding.

Epithelial/endothelial mesenchymal transition (EMT/EndMT) is an important pathogenic mechanism that occurs during renal fibrosis (21-23). EndMT is considered to be a special type of EMT that occurs during the transition of glomerular endothelial cells to mesenchymal cells (24). It is involved in tissue wound repair and remodeling under physiological conditions while promoting renal fibrosis under pathological conditions (25,26). In animal models of DN, multiple signaling pathways such as the TGF- β signaling pathway, Wnt signaling pathway, Hedgehog signaling pathway, Fibroblast growth factor receptors (FGFRs) 1 signaling pathway, and Sirtuin3 (SIRT3) signaling pathway are implicated in the regulation of EndMT during renal fibrosis (27). In addition, inhibiting the expression of proinflammatory factors, including tumor necrosis factor- α (TNF- α) and interleukin (IL)-6 and IL-1 β , in DN rats can slow down the progression of nephropathy (28). Among these numerous related pathological mechanisms, the present study focuses attention on the association between transforming growth factor- β 1 (TGF- β 1)/SMAD homolog (SMAD)3 signaling pathway and EMT, and whether Dapagliflozin plays a role in the treatment of DN through this mechanism.

TGF- β 1 is a multifunctional regulatory polypeptide that can control several cell activities, including cell proliferation, differentiation, and apoptosis (29). Additionally, it plays an important regulatory role in the development of DN (30). Studies have shown that Smad7 inhibits the TGF- β /Smad-mediated renal fibrosis signaling pathway by blocking the activation of Smad2/3. The TGF- β /Smad pathway participates in the pathogenesis of renal fibrosis (31). The TGF- β /Smad3 signal pathway has been shown to be highly activated in DN (32). Li *et al* (33) found that high concentrations of glucose in cell culture media activated the TGF- β /Smad pathway. Inhibition of the TGF- β 1/Smad signaling pathway can reduce renal fibrosis in diabetic rats (34). Numerous studies have also demonstrated that reducing EMT by inhibiting the TGF- β 1/Smad pathway can significantly reduce renal fibrosis and treat DN (35-37).

However, there are no reports on the contribution of the TGF- β /Smad signaling pathway in the involvement of Dapagliflozin treatment of renal fibrosis in T2DM rats. In addition, since the efficacy of metformin in the treatment of T2DM is well established, several relevant studies have used it as a positive control drug when empirically researching the efficacy of drugs. Therefore, metformin is also used as the positive control drug in the present study. Therefore, when designing the research scheme of the present study, the effect of the combined use of Dapagliflozin and metformin on renal fibrosis in T2DM rats was not assessed. Streptozotocin (STZ) was used to induce T2DM in the rat model, in order to explore the mechanism of Dapagliflozin on renal fibrosis in T2DM rats and provide a potentially novel direction for the development of therapeutics for the treatment of DN.

Materials and methods

Experimental animals. A total of 24 healthy SPF-grade male SD rats, weighing 180-220 g, were provided by the Experimental Animal Center of Shanghai Changzheng Hospital. They were reared in an environment with a relative humidity of 60%, a temperature of 22°C, and a 12 h light/dark cycle, with *ad libitum* access to water and food. Experiments were performed 1 week after acclimation. The present study was approved by the Shanghai Changzheng Hospital Animal Ethics Committee.

Grouping and drug intervention. A total of 24 rats were randomly divided into a normal (Control) group, Model group (STZ-induced T2DM rats), a Dapagliflozin (Dapa) group, and a metformin (Met) group, with 6 rats in each group. Rats in the control group were fed a normal diet, while those in the other groups were fed a high-glucose, high-fat diet for 4 weeks (28). After 4 weeks, the rats in each group were fasted for 12-16 h, and the Control group was intraperitoneally injected with the same amount of sodium citrate buffer. The other groups of rats were intraperitoneally injected with 35 mg/kg streptozotocin (STZ; Dalian Meilun Biology Technology Co., Ltd.). A week after STZ induction, the fasting blood glucose (FBG) of rats was >16.7 mmol/l, and the 24-h urine output was >150% of normal, suggesting that T2DM rat models were successfully constructed (19). After successful modeling, the Control and Model groups were given an equal amount of normal saline; the Dapa group was given 1 mg/kg Dapagliflozin (Dapagliflozin, AstraZeneca) (38,39) once a day for 4 weeks (40); the Met group was administered 200 mg/kg metformin (Squibb) once a day for 4 weeks (40). The FBG levels of these rats were tested once every week. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (35 mg/kg) after 4 weeks and peripheral blood was collected. The rats were subsequently sacrificed by cervical dislocation, and kidney tissues were removed for subsequent analysis.

Biochemical detection. Rat peripheral blood was taken, and the supernatant was collected after centrifugation at 1250 x g, at 4°C for 10 min. The blood urea nitrogen (BUN), serum creatinine (SCr), 24 h urine protein level, and glycosylated hemoglobin (HbA1c) were detected using corresponding assay kits (Dalian Meilun Biology

Technology Co., Ltd.). Total cholesterol (TC; cat. no. msw E2142), triglyceride (TG; cat. no. msw E2170), high-density lipoprotein cholesterol (HDL-c; cat. no. msw E2457) and low-density lipoprotein cholesterol (LDL-c; cat. no. msw E2171) in serum were measured to assess lipid levels in each group of rats according to the manufacturer's instructions (Dalian Meilun Biology Technology Co., Ltd.). Additionally, 24-h urinary albumin and creatinine excretion levels were measured, and albumin-to-creatinine ratios were calculated using rat urinary albumin (cat. no. QC12245; Shanghai Qincheng Biotechnology Co., Ltd.) and urinary creatinine (cat. no. BY-PD6160S; Shanghai Baiyi Biotechnology Co., Ltd.) ELISA kits. In addition, the serum levels of insulin (RC-R17363T, DRG International) and TGF- β 1 (mlsw E2400, Dalian Meilun Biology Technology Co., Ltd.) were measured using rat-specific ELISA kits.

Renal index tests. After the rat kidneys were separated and the water was wiped dry, the kidneys were weighed. The renal index was calculated as follows: Renal index=kidney weight/body weight.

Hematoxylin and eosin (H&E) staining and periodic acid-Schiff (PAS) staining. The renal tissue was fixed in 4% paraformaldehyde solution overnight at room temperature, dehydrated with an increasing gradient of alcohol solutions, and embedded in a paraffin block. The embedded tissue was cut into sections with a thickness of 3 μ m using a microtome. The sections were deparaffinized with xylene, stained with H&E (Mexin), then mounted with neutral resin, observed, and imaged under an optical microscope (x100 and x200 magnification; Olympus Corporation). As for PAS staining, the sections were stained to assess glomerular mesangial expansion. Briefly, the paraffin sections were incubated with 0.5% periodic acid for 5 min, rinsed with distilled water, incubated with Schiff reagent for 15 min at room temperature, washed in tap water for 5 min, counterstained with hematoxylin at room temperature for 1 min, mounted using neutral resin, observed, and imaged under an optical microscope (x100 and x200 magnification; Olympus Corporation). Image Pro Plus version 6.0 (Media Cybernetics, Inc.) was used to quantify the positively stained area of the glomerular.

Masson staining. Paraffin-coated renal tissue sections were deparaffinized and rehydrated with 100, 95, and 70% ethanol solutions. Next, they were stained with Regaud's hematoxylin at room temperature for 1 min and rinsed thoroughly with distilled water. Subsequently, the sections were stained in Masson's acid solution at room temperature (Fuzhou Maixin Biotech Co., Ltd.) for 10 min, washed with 2% glacial acetic acid, differentiated with 1% molybdenum phosphate at room temperature for 5 min, and then directly transferred to aniline blue solution (Fuzhou Maixin Biotech Co., Ltd.) at room temperature for 5 min. Subsequently, the sections were briefly rinsed in distilled water and differentiated in 0.2% glacial acetic acid. In the post-staining step, they were dehydrated with 95 and 100% ethanol solutions, washed with xylene, and fixed with neutral resin at room temperature for 5 min. Finally, the sections were observed and imaged under an optical microscope (x100 and x200 magnification; Olympus Corporation).

Western blotting. The rat renal tissues were lysed using RIPA Lysis Solution (Beyotime Institute of Biotechnology), centrifuged at 4°C at 13,400 \times g for 15 min, and the supernatant was collected. BCA protein assay reagent (Thermo Fisher Scientific, Inc.) was used to determine the total protein concentration. The proteins (30 μ g/lane) were loaded on a 10% SDS gel, resolved by SDS-PAGE, and transferred to PVDF membranes. Next, membranes were blocked in skimmed milk for 1 h at room temperature, the membranes were incubated overnight at 4°C with the primary antibodies which include: Anti- α smooth muscle Actin antibody (SMA; cat. no. ab124964; 1:1,000; Abcam), anti-Vimentin antibody (cat. no. ab92547; 1:1,000; Abcam), anti-E Cadherin antibody (cat. no. ab231303; 1:1,000; Abcam), anti-TGF- β 1 antibody (cat. no. ab215715; 1:1,000; Abcam), anti-MADH7/SMAD7 antibody (cat. no. ab216428; 1:1,000; Abcam), anti-phospho (p)-Smad3 antibody (cat. no. ab63403; 1:1,000; Abcam), and anti- β actin antibody (cat. no. ab115777; 1:5,000; Abcam). Subsequently, the membranes were washed three times with TBST, and then incubated with the secondary antibodies: Goat anti-mouse IgG H&L (HRP) (cat. no. ab205719; 1:5,000; Abcam) or goat anti-rabbit IgG H&L (HRP) (cat. no. ab205718; 1:5,000; Abcam) at room temperature for 1 h. Signals were visualized using a chemiluminescent solution, and the proteins were imaged in the exposure apparatus (41).

Statistical analysis. Data are presented as the mean \pm SD. SPSS version 21.0 (IBM Corp.) was used for data analysis. Differences between multiple groups were analyzed using a one-way ANOVA followed by a post hoc Tukey's test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Dapagliflozin improves renal functional impairment in T2DM rats. Firstly, the renal function indices of rats were tested to determine the effect of Dapagliflozin on the kidneys of T2DM rats. The results showed that during the 4 weeks of intragastric administration, the FBG levels in the Control group of rats were maintained at a normal level of \sim 7 mmol/l, while that in the Model group was >16.7 mmol/l (remaining in the high glucose range). Starting at 2 week, the FBG levels of the Dapa group and the Met group gradually decreased with the duration of medication, but the FBG levels of the Dapa group were lower than that of the Met group (Fig. 1A). Following 4 weeks of intragastric administration, the levels of HbA1c, BUN, SCr, and 24 h urine protein in the Model group were significantly higher than those in the Control group; while Dapagliflozin and metformin significantly reduced HbA1c, BUN, SCr, and 24 h urine protein levels in rats after STZ induction (Fig. 1B-E). These results indicate that Dapagliflozin has a protective effect on the kidneys of T2DM rats.

Dapagliflozin decreases lipid levels and increases insulin levels in T2DM rats. Subsequently, the effects of Dapagliflozin on lipid and insulin levels in T2DM rats. The results showed that the serum TC, TG, and LDL-c levels were significantly

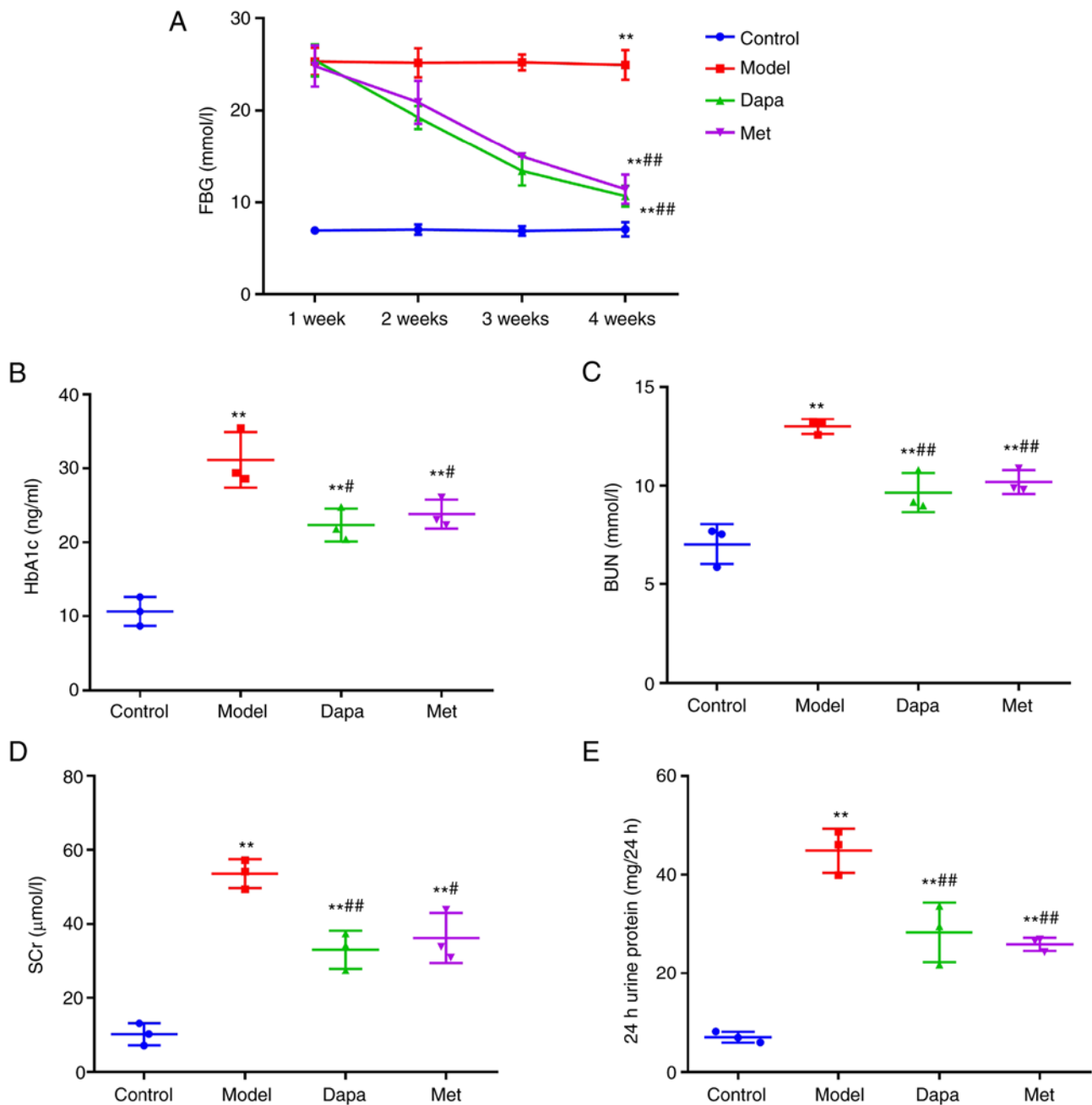


Figure 1. Effect of Dapagliflozin on rat serum biochemical parameters. (A) Serum FBG levels of rats in each group. (B) Biochemical detection of HbA1c levels in serum of rats after intragastric treatment. (C) Serum BUN levels of rats in each group. (D) Serum SCr levels of rats in each group. (E) The 24 h urine protein levels in the urine of rats in each group. $n=3$ per group. ** $P<0.01$ vs. Control group; # $P<0.05$; ## $P<0.01$ vs. Model group. FBG, fasting blood glucose; BUN, blood urea nitrogen; SCr, serum creatinine; Dapa, Dapagliflozin group; Met, metformin group.

increased, and the levels of HDL-c and insulin were markedly decreased in STZ-induced T2DM rats compared with the Control group. Compared with the Model group, treatment with Dapagliflozin and metformin significantly lowered the serum levels of TC, TG, and LDL-c and elevated the levels of HDL-c and insulin in T2DM rats (Fig. 2A-E). Accordingly, Dapagliflozin decreased the lipid levels while increasing insulin levels in T2DM rats.

Dapagliflozin improves renal tissue damage in T2DM rats. Next, the effect of Dapagliflozin on the morphology and structure of the renal tissue in diabetic rats was observed. The Model group showed a notably higher renal index of rats than

the Control group; the Dapa and Met groups displayed a much lower renal index than the Model group (Fig. 3A). According to the H&E staining results, the renal tissues of the Control group did not exhibit any significant pathological changes, and the histology of the glomeruli and renal tubules was normal. The renal tissues in the Model group showed degeneration and fibrosis, detached renal tubular epithelial cells, edema, glomerular mesangial cell proliferation, glomerular basement membrane thickening, renal tubular dilatation, and atrophy. In the Dapa and Met groups, the extent of renal disease was reduced, and the proliferation of glomerular mesangial cells was reduced (Fig. 3B). Thus, Dapagliflozin may reduce renal tissue damage in diabetic rats.

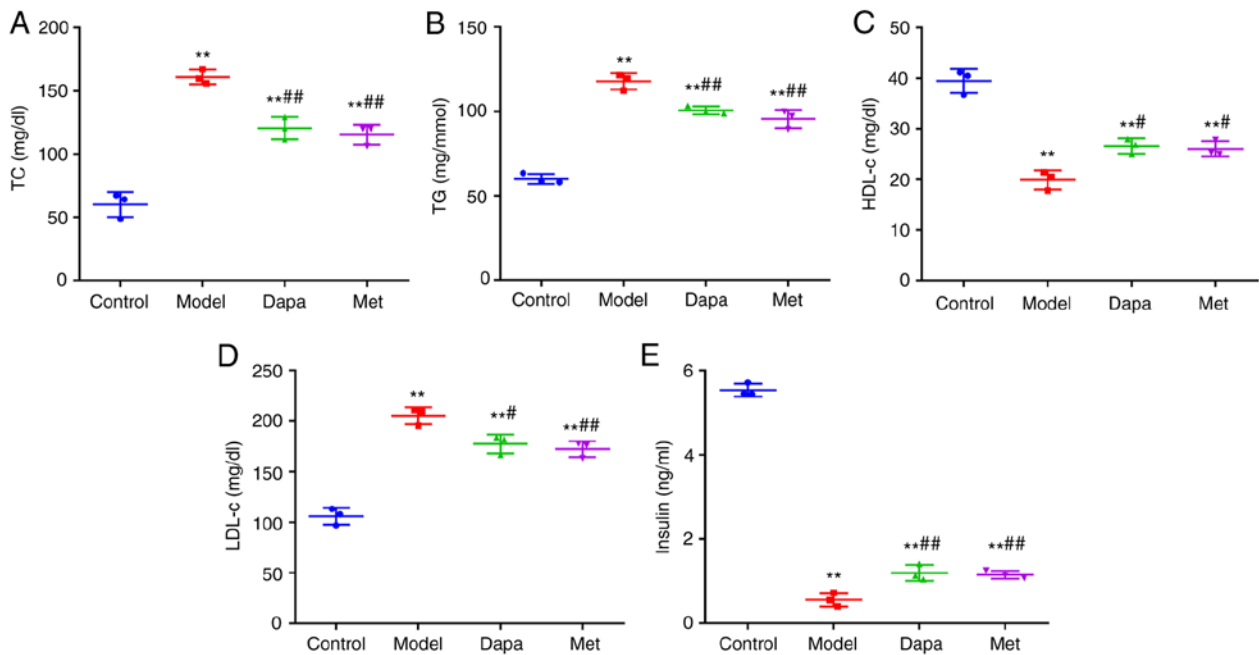


Figure 2. Dapagliflozin decreases the lipid levels and increases the insulin levels in T2DM rats. ELISA was performed to measure the levels of serum (A) TC, (B) TG (C) HDL-c, (D) LDL-c, and (E) insulin. N=3 per group. **P<0.01 vs. Control group; #P<0.05 and ##P<0.01 vs. Model group. TC, total cholesterol; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein.

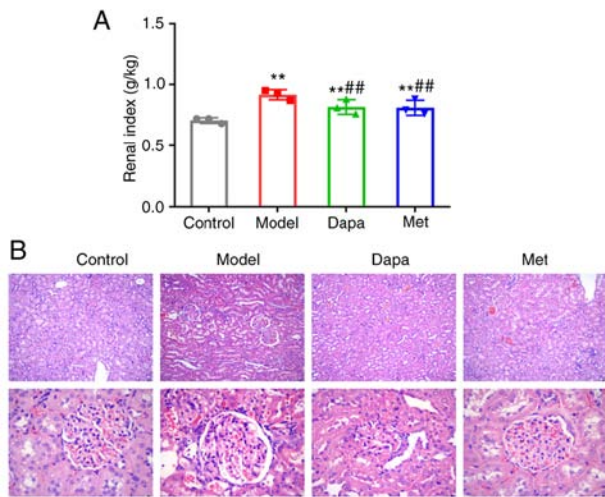


Figure 3. Effect of Dapagliflozin on rat kidney histomorphology. (A) Comparison of rat renal indexes in each group. (B) Hematoxylin and eosin staining showing renal damage in the tissues. N=3 per group. **P<0.01 vs. Control group; ***P<0.01 vs. Model group. Dapa, Dapagliflozin group; Met, metformin group.

Dapagliflozin reduces renal fibrosis in T2DM rats. Moreover, the effect of Dapagliflozin on renal fibrosis in diabetic rats was studied. Masson staining results showed that the glomerulus, renal tubules, and interstitial collagen deposition in the Control group were physiologically normal. However, in the Model group, the glomerular basement membrane was notably thicker, collagen deposition in the renal interstitium increased, and the basement membrane of certain renal tubules was also thicker, accompanied by a degree of dilation and vacuole-like lesions. As for the Dapa and Met groups, the collagen deposition in glomeruli and interstitial regions was significantly lower

compared with the Model group (Fig. 4A). With respect to PAS staining results, glomerular mesangial matrix deposition and the degree of renal fibrosis were notably increased in the Model group; after treatment with Dapagliflozin and Metformin, glomerular mesangial matrix deposition and the renal fibrosis in T2DM rats were effectively improved (Fig. 4B-D). In addition, compared with the Control group, the expression levels of renal fibrosis-related proteins α -SMA and Vimentin in the renal tissue of the Model group were significantly increased, while the expression levels of E-cadherin were remarkably reduced. As opposed to the Model group, a notable drop in the expression levels of α -SMA and Vimentin and a significant increase in the expression levels of E-cadherin were observed in the kidney tissues of rats in the Dapa group and Met groups (Fig. 4E). This shows that Dapagliflozin inhibited kidney fibrosis in diabetic rats.

Dapagliflozin inhibits the activation of the TGF- β 1/Smad signaling pathway in T2DM rats. Finally, to explore the molecular mechanism by which Dapagliflozin exerted its protective effects on the kidneys of diabetic rats, the expression of TGF- β 1/Smad signaling pathway-related proteins was determined. ELISA results revealed that the serum levels of TGF- β 1 were significantly higher in STZ-induced T2DM rats compared with the Control rats, and were significantly lower in the Dapa and Met groups compared with the Model group (Fig. 5A). In terms of western blotting results, STZ induced a significant increase in the protein expression levels of TGF- β 1 and p-Smad3 as well as a significant decrease in the expression of Smad7 protein in rat kidney tissues. Compared with the Model group, the Dapa and Met groups exhibited significantly reduced expression levels of TGF- β 1 and p-Smad3, and increased expression levels of Smad7 protein in the kidney tissues. In addition, the protein expression

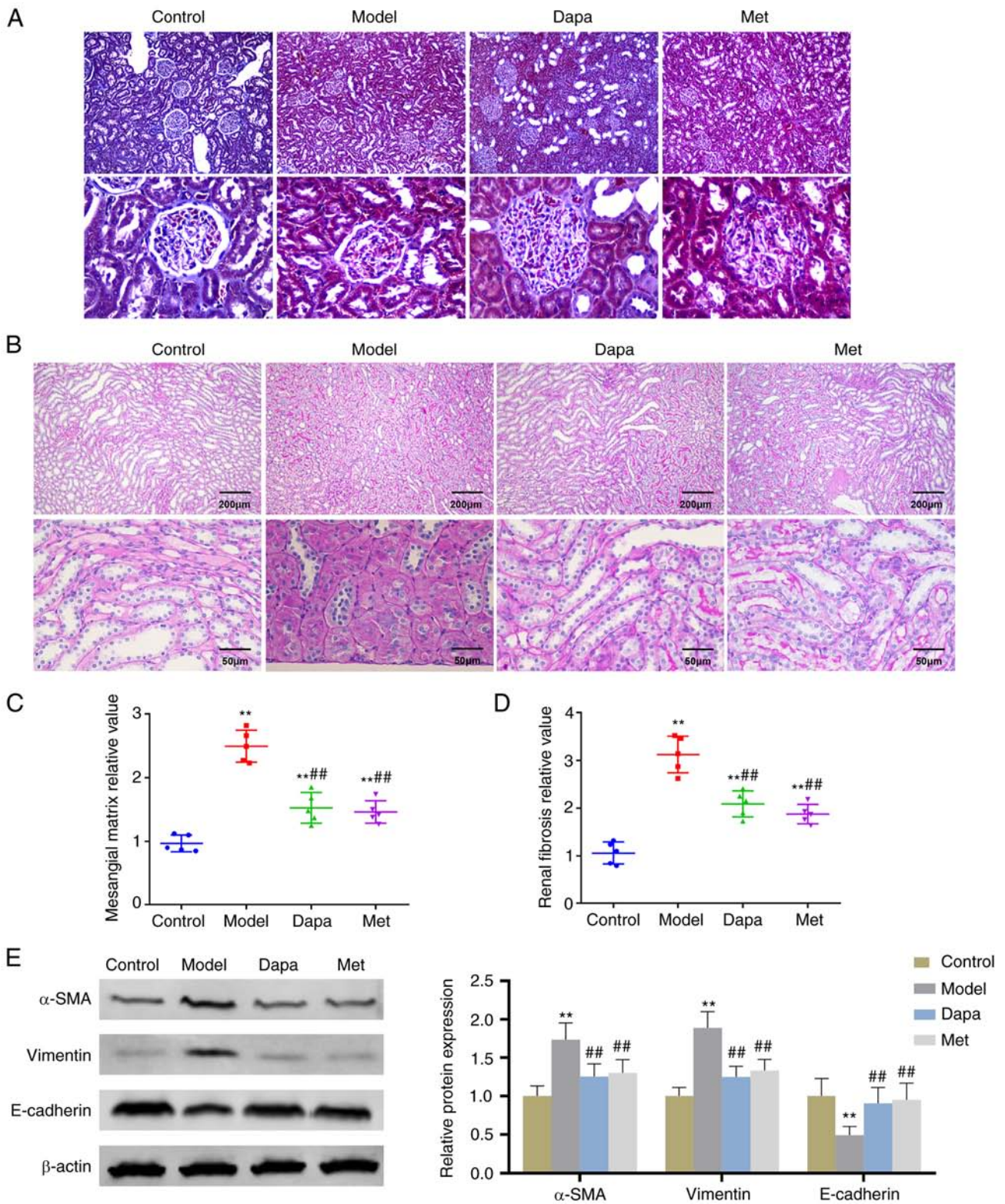


Figure 4. Effect of Dapagliflozin on renal fibrosis in rats. (A) The degree of fibrosis in rat kidney tissues was observed by Masson staining. (B-D) The degree of glomerular mesangial matrix and renal fibrosis was calculated by Periodic Acid-Schiff staining. $n=5$. (E) The protein expression levels of α -SMA, vimentin, and E-cadherin in rat kidney tissues were detected by western blotting. $n=3$. ** $P<0.01$ vs. Control group, ## $P<0.01$ vs. Model group. α -SMA, α -smooth muscle actin.

levels of total Smad3 were not significantly altered in the tissues of rats in each group (Fig. 5B). Together, these results showed that Dapagliflozin may protect the renal function of diabetic rats by inhibiting the activity of the TGF- β 1/Smad signaling pathway.

Discussion

Diabetes mellitus, resulting from complete or reduced insulin secretion and/or insufficient insulin action, is characterized by chronic hyperglycemia and impaired metabolism

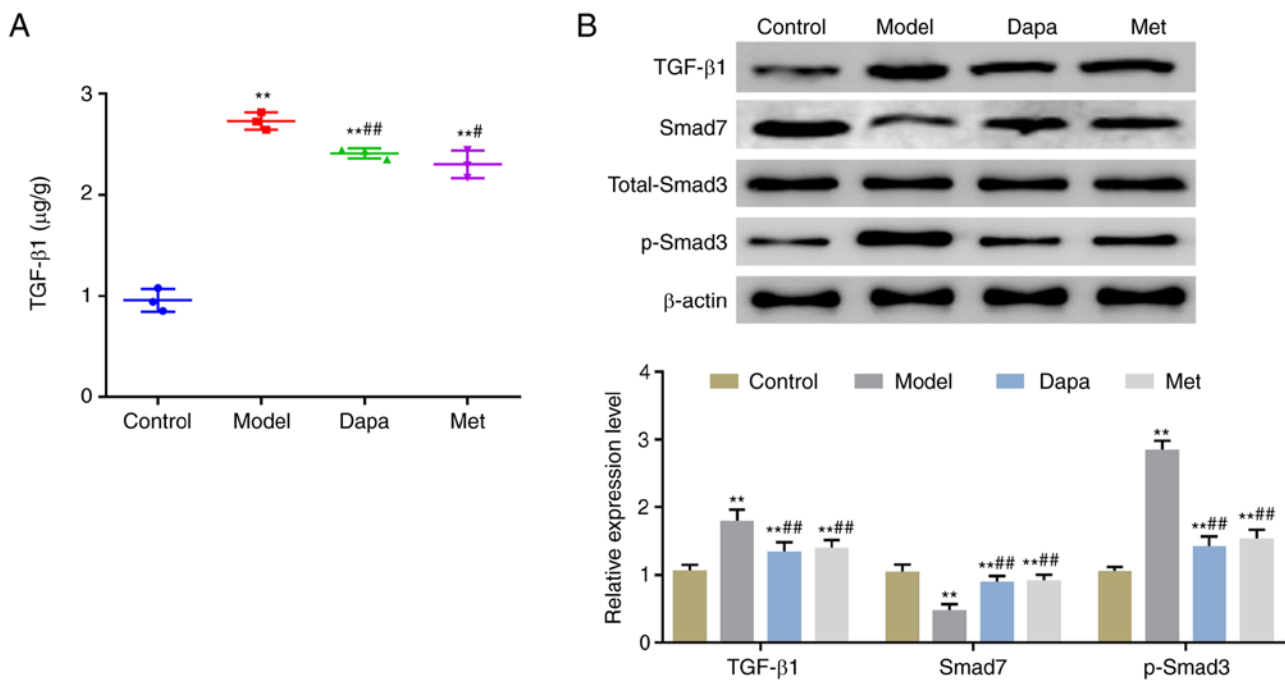


Figure 5. Effect of Dapagliflozin on the TGF- β 1/Smad signaling pathway in rat renal tissues. (A) The levels of TGF- β 1 in the serum of each group of rats was detected by ELISA. (B) The protein expression levels of TGF- β 1, Smad7, p-Smad3, and total Smad3 in the kidney tissues of rats in each group were detected by western blotting. n=3. **P<0.01 vs. Control group, #P<0.05 and ##P<0.01 vs. Model group. p-, phospho.

of carbohydrates, lipids, and proteins. T2DM is the most common form of diabetes, accounting for 90-95% of all diabetic patients (42). In China, diabetes and prediabetes are more prevalent among individuals >20 years old, with 15.5% of these instances being T2DM (43). The most common cause of CKD, DN, can lead to end-stage renal disease and even patient death (44,45). Therefore, it is crucial to treat DN as soon as possible. Dapagliflozin, as an anti-diabetic drug, is currently used clinically to treat DN. Studies have revealed several effects of Dapagliflozin, such as reducing the risk of renal endpoints by 47%, lowering the weight and blood pressure of DN patients, and improving blood glucose and urine protein levels (46). If abnormalities are seen in serum BUN, SCr, and 24 h urine protein levels, which are currently used to evaluate renal function, this indicates that diabetes may cause nephropathic complications (47). In the present study, T2DM rat models were established by intraperitoneal injection of 35 mg/kg STZ. It was found that the levels of FBG, HbA1c, BUN, SCr, 24 h urine protein, and lipids, as well as the renal index of T2DM rats increased significantly, whereas insulin levels decreased significantly. Additionally, the proliferation of glomerular mesangial cells, the thickening of the glomerular basement membrane, the dilatation and atrophy of renal tubules, accompanied by a certain degree of dilatation, and vacuole-like lesions were visible in the renal tissues. However, renal injury, lipid levels, and insulin levels were effectively improved in T2DM rats after Dapagliflozin treatment. It is evident that Dapagliflozin can protect rats from STZ-induced T2DM.

The pathogenesis of DN is complicated. Renal interstitial fibrosis is a common route from the development of T2DM nephropathy to end-stage renal disease, which is primarily caused by the excessive accumulation of extracellular matrix

(ECM) in the renal interstitium. This results in a decrease in the expression of epithelial cell markers such as E-cadherin and an increase in the expression of interstitial cell markers such as α -SMA and Vimentin. Under normal circumstances, fibrocytes do not express α -SMA, so α -SMA expression levels are often used as an indicator of fibrosis (48).

In the present study, it was found that STZ induced significant thickening of the glomerular basement membrane in the renal tissue of T2DM rats, collagen deposition in the renal interstitium, and thickening of the tubular basement membrane. Additionally, the expression levels of α -SMA and Vimentin increased significantly in the renal tissue and the E-cadherin expression levels decreased significantly. It follows that STZ can induce renal fibrosis in rats. After treatment with Dapagliflozin, the pathological changes such as renal fibrosis and collagen deposition in the renal interstitium of T2DM rats were alleviated; at the same time, the protein expression levels of α -SMA and vimentin in rat renal tissues were significantly reduced, whereas the protein expression levels of E-cadherin increased significantly. Collectively, Dapagliflozin can effectively alleviate renal tissue fibrosis in T2DM rats.

Further, the molecular mechanism through which Dapagliflozin improved renal fibrosis in T2DM rats was explored. TGF- β 1 is involved in the growth and differentiation of various cells such as hepatocytes, renal cells, and cardiac cells, as well as in the synthesis and accumulation of extracellular matrix, which can lead to renal interstitial fibrosis and even glomerulosclerosis (49). Moreover, TGF- β 1 promotes fibroblast transformation and ECM synthesis by regulating matrix metalloproteinases and inhibits ECM decomposition. Subsequently, it not only leads to podocyte apoptosis, basement membrane exfoliation, and protein leakage, it also reduces the number of nephrons and causes glomerulosclerosis (50).

Hyperglycemia has been demonstrated to induce an increase in the expression of TGF- β 1 in mesangial cells, thereby resulting in the accumulation of extracellular matrix (51). Smad protein is the transduction molecule of the TGF- β family signal from the receptor to the nucleus, and at present, it is hypothesized that Smad is the only substrate of TGF- β , and imbalances in the expression are the molecular basis of renal fibrosis (52,53). The Smad-dependent signaling pathway is a classical signaling pathway by which TGF- β 1 induces fibrosis (52,53). Smad3 is an activated Smad protein and a downstream regulator of TGF- β 1. Increased expression of Smad in DN animal models indicates that Smad3 is crucial to the development of DN fibrosis. The concentration of Smad3 in the fibrotic kidney model is significantly higher than in normal tissues (54,55). Smad7, an inhibitory Smad protein, can compete with Smad2 or Smad3 to bind to the TGF- β receptor I to inhibit Smad2/Smad3 phosphorylation and its translocation to the nucleus, and increase ubiquitin-mediated degradation of TGF- β receptor I. As a joint result of these actions, it negatively regulates the TGF- β 1/Smad pathway. It has been recognized that Smad7, as an endogenous TGF- β 1 antagonist, inhibits the TGF- β 1/Smad signaling pathway (56) and reduces the progression of experimental renal fibrosis (57). In the present study, it was found that the expression levels of TGF- β 1 and p-Smad3 in the kidney tissue of T2DM rats were significantly increased, and the expression levels of Smad7 were considerably reduced. After treatment with Dapagliflozin, the protein expression levels of TGF- β 1 and p-Smad3, conversely, decreased significantly while the protein expression levels of Smad7 increased significantly in the kidney tissue of rats induced with STZ. Briefly, the TGF- β 1/Smad signaling pathway may be one of the mechanisms by which Dapagliflozin reduces renal fibrosis.

The novelty of the present study is that it was demonstrated for the first time that Dapagliflozin alleviates renal fibrosis in T2DM rats by inhibiting the TGF- β 1/Smad signaling pathway and has a protective effect on STZ-induced T2DM rats. However, this study lacked simultaneous *in vitro* observations and validation through other phenotypic rescue experiments, and only observed changes in indicators under the actions of drugs. The present study is a preliminary observational experiment. To confirm the findings of the results, further mechanistic explanations are required in future studies. Secondly, in the animal intervention experiments, the effect of the combined use of Dapagliflozin and Metformin on DN was not explored, and a non-disease group only treated with Dapagliflozin was not included to exclude any potential effects of Dapagliflozin on the normal health status. Additionally, whether Dapagliflozin exerts its therapeutic effect in a dose-dependent manner has also not been explored. STZ has significant defects in the construction of DN models due to its islet destruction and nephrotoxic effects. In view of this, there is an urgent need to identify more reliable modeling methods to compensate for these deficiencies. Finally, the present study did not fully detect all physiological indicators in the rats. It is necessary to include indicators such as blood glucose, blood lipids, blood uric acid, and urine protein (albumin excretion rate and creatinine clearance rate) to better evaluate changes in liver and kidney function.

In summary, Dapagliflozin can improve STZ-induced renal fibrosis in T2DM rats, and its mechanism may be related to the reduced activity of the TGF- β 1/Smad signaling pathway.

This finding provides a theoretical basis for the medicinal use of Dapagliflozin. However, further study is required to understand how Dapagliflozin affects the TGF-1/Smad signaling pathway and treat DN.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SX, YL, and WT conceived and designed the study, and wrote the manuscript. YL and XL analyzed the data. SX and WT collected and provided the samples for the study. SX and WT confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Shanghai Changzheng Hospital Animal Ethics Committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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