European Review for Medical and Pharmacological Sciences 2017; 21: 867-872

Glipizide blocks renal interstitial fibrosis by inhibiting AKT signaling pathway

G. YANG, G. ZENG, J.-P. WU, O. JIANG, Y.-B. ZENG, S.-J. HUANG, J.-J. HUANG, D.-Q. WU

Clinical Laboratory, Affiliated Second People's Hospital of Luzhou Medical College, Neijiang City, Sichuan, China

Abstract. – OBJECTIVE: Diabetes affects the renal function at a certain stage. Oral medication glipizide plays a hypoglycemic effect mainly through releasing insulin, while more insulin is derived from islet β cells. It is still controversy whether antidiabetics. This study mainly intends to investigate the role of glipizide in inhibiting renal interstitial fibrosis.

MATERIALS AND METHODS: A total of 93 SD rats were purchased from Guangdong animal monitoring and established unilateral ureteral obstruction (UUO) model to simulate renal interstitial fibrosis. Forty rats in the experimental group received glipizide intraperitoneal injection for a week at 30 days after modeling, while another 40 rats in the control group received a normal saline injection. The last 10 rats were treated as blank group. Hematoxylin and eosin (HE) staining was applied to test renal interstitial fibrosis. Immunohistochemistry was used to detect fibronectin expression in glomerular and renal tubules. AKT signaling pathway related factors expression was measured by Western blot to determine AKT signal activation.

RESULTS: HE staining showed that the entire kidney cytoplasm red dye becomes shallow, renal medulla gradually disappears, renal tubular epithelial cells enlarge, vacuoles degeneration, renal tubule and collecting tube expansion, inflammatory cells infiltration after UUO modeling. Glipizide treatment decreased dilated renal tubule number, improved glomerulus integrity, and reduced inflammatory infiltration. Fibronectin level in the experimental group was significantly lower than that in control (p<0.05). Western blot revealed that p-AKT expression downregulated after glipizide treatment.

CONCLUSIONS: Glipizide blocks renal interstitial fibrosis by inhibiting AKT signaling pathway.

Key Words:
Glipizide, Renal interstitial fibrosis, AKT.

Introduction

Glipizide, a type of sulfonylurea, is used in the treatment of type 2 diabetes since the 1950s. It is the second-generation sulfonylureas oral hypoglycemic agent as it can stimulate insulin secreted by β cells[1,2], especially promote glucose-stimulated insulin secretion. Until 1995, sulfonylurea drugs were still the only oral hypoglycemic agent that can be used in the treatment of type 2 diabetes, and more than 50% of diabetic patient treatment was associated with sulfonylurea[3,4]. Recent studies[5,6] revealed that diabetics have a higher risk of suffering from colorectal cancer, liver cancer, pancreatic cancer, and prostate cancer. As is known to all, the renal interstitial fibrosis is a chronic disease, which may lead to the tumor. Epidemiological studies[7,8] showed that long-term usage of the hypoglycemic drug could reduce markedly the tumor occurrence. It was reported that glipizide could significantly inhibit the vascular formation and inhibit tumor growth and metastasis by upregulating NPRA level on breast cancer model mice and subcutaneous transplantation tumor model mice. It suggested that glipizide might be treated as an inhibitor to suppress unlimited cellular proliferation[9].

Renal disease occurrence is closely related to diabetes. The study aiming to evaluate aliskiren impact on renal tubular function and renal interstitial fibrosis status in patients with diabetic nephropathy presented that aliskiren injection can regulate related factors in urine and serum, and elevate albumin reabsorption rate of renal tubules to improve effectively the renal tubular function and the renal interstitial fibrosis status. It was also revealed that benazepril may suppress hyperglycemia and advanced glycation end products (AGEs) to inhibit connective tissue growth factor (CTGF) expression in the renal tissue of diabetic rats, sug-

Corresponding Author: Daoquan Wu, MD; e-mail: daoquanwuzxcc@sina.com

gesting that blocking CTGF can effectively delay diabetic nephropathy progression. Tumor occurrence is a gradual process of evolution, and the occurrence of renal cancer is from renal tubule cells and medulla cells fibrosis. Glipizide primarily plays a hypoglycemic effect through releasing insulin, while insulin is mainly from β cells. There is still a lack of reports about the relationship between renal fibrosis and glipizide. For renal fibrosis is the early development phase of renal tumor, and glipizide could be used to treat the tumor. We aimed to explore whether glipizide can inhibit tumor through improving renal interstitial fibrosis.

Materials and Methods

Renal Interstitial Fibrosis Modeling

A total of 93 SD rats were purchased from Luzhou Medical College to construct renal interstitial fibrosis model using unilateral ureteral obstruction method. The rat was anesthetized and fixed to expose the abdomen. After alcohol disinfection, the ureter was isolated and ligated by two 5-0 silk threads (Jetway Biotech Co., Ltd, Guangzhou, China). The upper was at the level of left inferior pole of the kidney. Then, the ureter was clipped between two ligations and the abdomen was closed. After 14 days, forty SD rats received an intraperitoneal injection of glipizide, continued for one week. HE staining was applied to test the renal interstitial fibrosis. Another 40 rats received normal saline as control. The last 10 rats received no treatment and were used as blank group.

Rats were used for all experiments, and all the procedures were approved by the Animal Ethics Committee of Affiliated Second People's Hospital of Luzhou Medical College.

HE Staining

The sample was fixed in formalin overnight. After dehydration, the tissue was embedded and cooled at 4°C. After repair, the tissue was sliced at 4 μm and baked at 65°C for 1 h. Next, the section was dewaxed and stained with hematoxylin and eosin. Lastly, the section was observed under a microscope and diagnosed by three different pathologists.

Immunohistochemistry

The sample was fixed in formalin overnight. After dehydration, the tissue was embedded and cooled at 4°C. After repair, the tissue was sliced at 4 μm and baked at 65°C for 1 h. After dewax, the section was washed with PBS for three times and repaired in sodium citrate (pH = 6.0) for 5 min. Then, the section was treated with 0.3% hydrogen peroxide at room temperature for 30-60 min. Next, the section was blocked with 10% fetal calf serum for 30 min and incubated with primary antibody overnight. The section was further incubated in secondary antibody at 37°C for 1 h and developed by DAB. After washed with PBS for three times, the section was redyed by hematoxylin for 3 min and sealed for photograph.

Western Blot

The tissue protein was extracted using the kit (Beyotime, Shanghai, China) and added with radioimmunoprecipitation assay (RIPA). After quantified using bicinchoninic acid (BCA) assay kit (Thermo Fisher, Waltham, MA, USA), the protein was boiled and centrifuged. The protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane. After washed with tris buffered saline-tween (TBS-T), the membrane was blocked by 5% skim milk for 1 h. Next, the membrane was incubated with primary antibody overnight and then incubated with secondary antibody at room temperature for 1 h. The membrane was further incubated with luminous fluid for 5 min and exposed on X-ray.

Statistical Analysis

All data analysis was performed by SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The data was compared by *t*-test. Each experiment was repeated for at least three times. $p<0.05$ was considered statistically significant.

Results

Glipizide Alleviated Renal Interstitial Fibrosis

It was found that glipizide improved renal interstitial fibrosis process. HE staining showed that compared with control, unilateral ureteral obstruction (UUO) model for 7 days and 14 days appeared renal cytoplasm red staining shallow. On the 7th day, renal medulla gradually disappeared and became large, vacuoles degeneration, partial renal tubule and collecting tube expansion, and inflammatory cells infiltration. Glipizide treatment decreased dilated renal tubule number, improved glomerulus integrity, and reduced inflammatory infiltration. On the 14th day, the above-mentioned phenomena aggravated, as the kidney in control presented significantly renal inter-

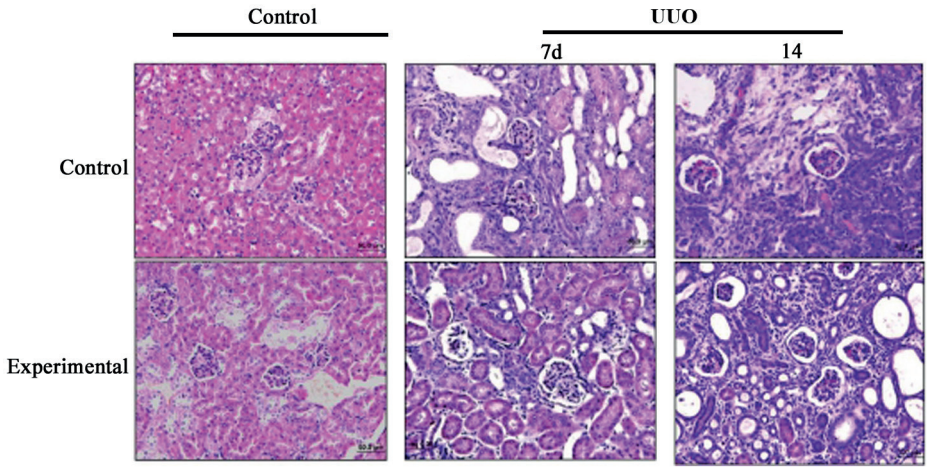

Figure 1. HE staining of renal interstitial fibrosis.

stitial fibroblasts hyperplasia, renal tubular necrosis, and glomerular number reduced. The pathological change in the experimental group was less than that in control (Figure 1).

Glipizide Reduce Fibronectin Expression

Fibronectin, known as a type of sugar molecule protein, mainly plays a role in cell adhesion. It is well known that fibronectin has an inseparable relationship with tissue fibrosis. Therefore, we used immunohistochemistry to detect fibronectin expression in the glomerulus and renal tubule. Compared with control, fibronectin was largely expressed in the model group. Glipizide treatment significantly decreased fibronectin expression and alleviated glomerular damage (Figure 2). Fibronectin in renal tubule also presented similar results (Figure 3).

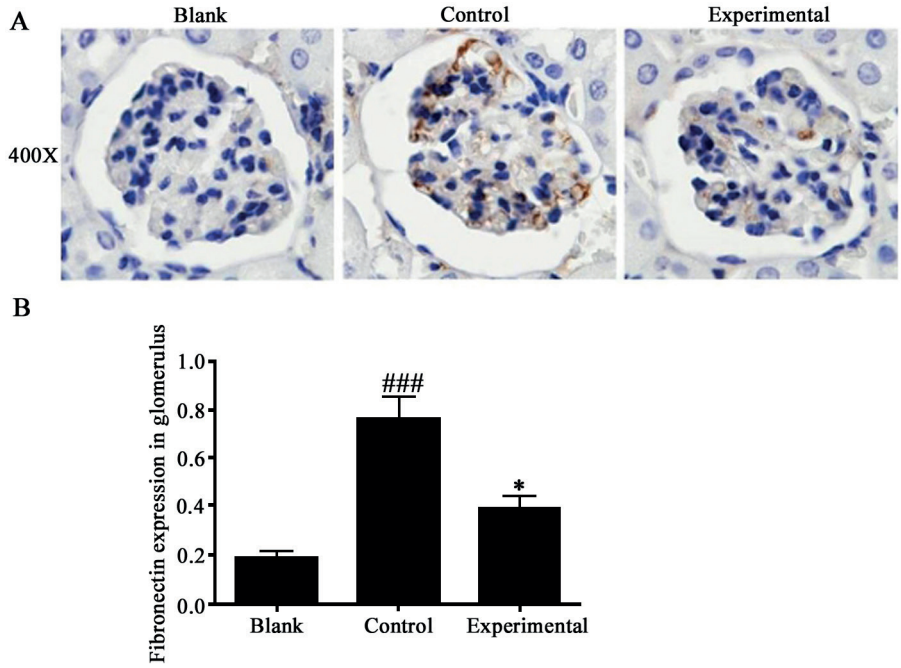

Figure 2. Fibronectin expression in glomerulus, *p<0.05.

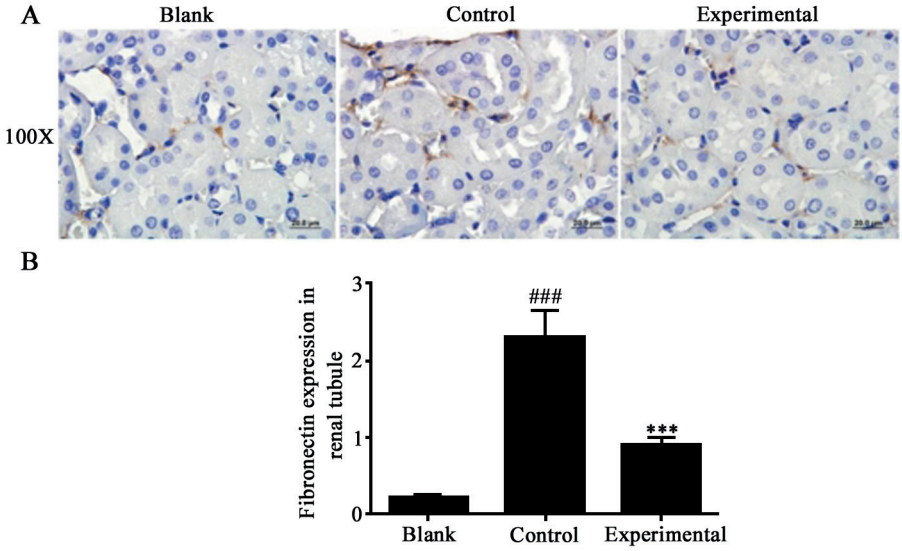

Figure 3. Fibronectin expression in renal tubule, $^{***}p<0.001$.

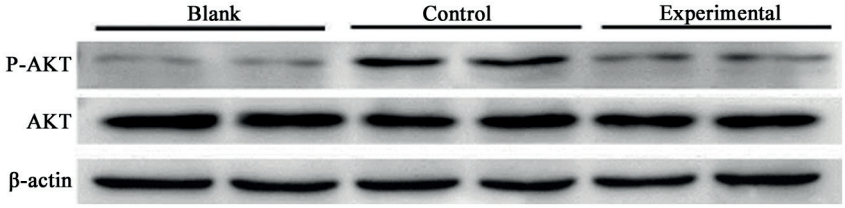

Figure 4. Glipizide downregulated p-AKT expression.

Glipizide Treatment Blocked AKT Signaling Pathway

Fibronectin plays an important role in the extracellular matrix, while the later mainly affects AKT signaling pathway. Western blot revealed that glipizide markedly downregulated p-AKT expression in renal tissue, suggesting that glipizide can inhibit AKT signaling pathway through downregulating fibronectin expression (Figure 4).

Discussion

Following the improvement of the quality of life, an increasing number of diabetes patients need better treatment. Some patients are also suffered from diabetic nephropathy, which seriously affects the normal life activities. Chronic renal disease may develop to renal interstitial fibrosis, and further may become a renal tumor, and the drug intervention plays a decisive role in this process. Common hypoglycemic drugs mainly focused on promoting insulin secreted by β cells[10-12]. As the most common medication, glipizide is also reported to play an intervention effect on tumor except diabetes treatment. The study used glipizide to treat genetic model rat and got remarkable effect[9]. We suggest that as the tumor is a gradual process, we can perform an intervention before the tumor occurrence. Therefore, we established rat UUO renal interstitial fibrosis model and found that glipizide treatment could improve the pathological process of renal fibrosis, while its mechanism still needs further investigation. It was found that as an important indicator of fibrosis, fibronectin played a decisive role in fibrosis. Fibronectin amount decides the degree of fibrosis[13-15]. Our results revealed that glipizide treatment significantly downregulated fibronectin expression.

Research showed that as an adhesion molecule, fibronectin mainly expressed in the extracellular

matrix. Extracellular matrix mostly affects AKT signaling pathway[16-19]. AKT is associated with multiple tumors regulation by affecting cell proliferation and programmed cell death, etc.[20]. Western blot demonstrated that glipizide treatment markedly reduced p-AKT expression compared with control, suggesting that glipizide could inhibit AKT signaling pathway through reducing fibronectin expression.

Conclusions

Our study showed that glipizide could retard tumor occurrence in early stage through inhibiting AKT signaling pathway to slow down renal interstitial fibrosis. Other related regulating mechanism still needs further in-depth investigation.

Acknowledgments

This study was supported in part by the Sichuan Provincial Department of Science and Technology of China (grant no. 15089); Sichuan province Department of medical association scientific research subject of China (grant no. S16043) and youth innovation of scientific research subject of Sichuan province medical association of China (grant no. Q16044).

Conflict of interest

The authors declare no conflicts of interest.

References

1) Mizuno CS, Chittiboyina AG, Kurtz TW, Pershadsingh HA, Avery MA. Type 2 diabetes and oral antihyperglycemic drugs. Curr Med Chem 2008; 15: 61-74.
2) Riddle MC. Oral pharmacologic management of type 2 diabetes. Am Fam Physician 1999; 60: 2613-2620.
3) Skyler JS. Diabetes mellitus: pathogenesis and treatment strategies. J Med Chem 2004; 47: 4113-4117.
4) Rodriguez C, Patel AV, Mondul AM, Jacobs EJ, Thun MJ, Calle EE. Diabetes and risk of prostate cancer in a prospective cohort of US men. Am J Epidemiol 2005; 161: 147-152.
5) Seow A, Yuan JM, Koh WP, Lee HP, Yu MC. Diabetes mellitus and risk of colorectal cancer in the Singapore Chinese Health Study. J Natl Cancer Inst 2006; 98: 135-138.
6) Huxley R, Ansary-Moghaddam A, Berrington de Gonzalez A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer 2005; 92: 2076-2083.
7) Inoue M, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. Arch Intern Med 2006; 166: 1871-1877.
8) Rousseau MC, Parent ME, Pollak MN, Siemiatycki J. Diabetes mellitus and cancer risk in a population-based case-control study among men from Montreal, Canada. Int J Cancer 2006; 118: 2105-2109.
9) Qi C, Zhou Q, Li B, Yang Y, Cao L, Ye Y, Li J, Ding Y, Wang H, Wang J, He X, Zhang Q, Lan T, Lee KK, Li W, Song X, Zhou J, Yang X, Wang L. Glipizide, an antidiabetic drug, suppresses tumor growth and metastasis by inhibiting angiogenesis. Oncotarget 2014; 5: 9966-9979.
10) Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 2005; 54: 533-539.
11) Chen CT, Lin J, Li Q, Phipps SS, Jakubczak JL, Stewart DA, Skripchenko Y, Forry-Schaudies S, Wood J, Schnell C, Hallenbeck PL. Antiangiogenic gene therapy for cancer via systemic administration of adenoviral vectors expressing secretable endostatin. Hum Gene Ther 2000; 11: 1983-1996.
12) Mehta MP, Paleologos NA, Mikkelsen T, Robinson PD, Ammirati M, Andrews DW, Asher AL, Burri SH, Cobbs CS, Gaspar LE, Kondziolka D, Linskey ME, Loeffler JS, McDermott M, Olson JJ, Patchell RA, Ryken TC, Kalkanis SN. The role of chemotherapy in the management of newly diagnosed brain metastases: a systematic review and evidence-based clinical practice guideline. J Neurooncol 2010; 96: 71-83.
13) Berk BC, Fujiwara K, Lehoux S. ECM remodeling in hypertensive heart disease. J Clin Invest 2007; 117: 568-575.
14) Bourdoulous S, Orend G, MacKenna DA, Pasqualini R, Ruoslahti E. Fibronectin matrix regulates activation of RHO and CDC42 GTPases and cell cycle progression. J Cell Biol 1998; 143: 267-276.
15) Barilla ML, Carsons SE. Fibronectin fragments and their role in inflammatory arthritis. Semin Arthritis Rheum 2012; 29: 252-265.
16) Carnesecchi S, Deffert C, Donati Y, Basset O, Hinz B, Preynat-Seauve O, Guichard C, Arbiser JL, Banfi B, Pache JC, Barazzone-Argiroffo C, Krause KH. A key role for NOX4 in epithelial cell death during development of lung fibrosis. Antioxid Redox Signal 2011; 15: 607-619.
17) Gooch JL, Barnes JL, Garcia S, Abboud HE. Calcineurin is activated in diabetes and is required for glomerular hypertrophy and ECM accumulation. Am J Physiol Renal Physiol 2003; 284: F144-154.

18) Gooch JL, Gorin Y, Zhang BX, Abboud HE. Involvement of calcineurin in transforming growth factor-beta-mediated regulation of extracellular matrix accumulation. J Biol Chem 2004; 279: 15561-15570.

19) Gorin Y, Block K, Hernandez J, Bhandari B, Wagner B, Barnes JL, Abboud HE. Nox4 NAD(P)H oxidase mediates hypertrophy and fibronectin expression in the diabetic kidney. J Biol Chem 2005; 280: 39616-39626.

20) Blaustein M, Perez-Munizaga D, Sanchez MA, Urrutia C, Grande A, Risso G, Srebrow A, Alfaro J, Colman-Lerner A. Modulation of the Akt pathway reveals a novel link with PERK/eIF2alpha, which is relevant during hypoxia. PLoS One 2013; 8: e69668