

Renoprotective Effects of Metformin and Its Relationship with Oxidative Stress in Type 2 Diabetic Mice

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Research Article

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Abstract

Background: Oxidative stress has previously been shown to play critical roles in the development of diabetes and its complications. The purpose of this research was to observe the reno-protective effect of metformin and its effect on oxidative stress in type 2 diabetic mice renal tissue.

Methods: Type 2 diabetes mellitus mice model was established by High-fat feed combined with small-dose STZ and randomly divided into diabetes model group, Metformin [MET, 250mg/(kg.d)] group, Glibenclamide (GLIB) [GLIB, 2.5mg/(kg.d)] group, and normal control group (NC). After 8 weeks of intervention, blood and urine samples were collected for detection of FBG, HbA1c, urine albumin (Alb), retinol-binding protein (RBP), podocalyxin (PCX), 8-OHdG, 8-iso-PG, and creatinine (Cr). Renal tissue specimens were preserved for observing renal glomerular basement membrane thickness (GBMT) and foot process fusion rate (FPFR) under electron microscopy.

Results: Compared with the NC group, FBG, HbA1c, urinary Alb/Cr (UACR), RBP/Cr (URCR), PCX/Cr (UPCR), 8-OHdG /Cr (UOHCR), and 8-iso-PG /Cr (UISOCR) significantly increased in the T2DM group ($P < 0.05$). Compared with the T2DM group, FBG, HbA1c, UACR, URCR, UPCR, UOHCR, and UISOCR were significantly reduced in the GLIB group and MET group ($P < 0.05$). Compared to the GLIB group, UACR, URCR, UPCR, UOHCR, and UISOCR decreased in the MET group ($P < 0.05$), but FBG and HbA1c were not differenced statistically between the two groups. GBMT and FPFR increased in the T2DM group ($P < 0.05$), which were reduced in the MET group and lighter than those in the GLIB group ($P < 0.05$).

Conclusion: Metformin intervention can play a reno-protective effect in type 2 diabetic mice, which may be related to its effect in inhibiting enhanced oxidative stress in vivo.

Introduction

The prevalence of diabetes has increased significantly in the past few decades, and it has become one of the most important health problems in the world. According to the International Diabetes Federation, the number of people with diabetes worldwide has increased to 592 million by 2035^[1]. Diabetic kidney disease (DKD) is one of the most important complications of diabetes and can cause end-stage renal disease in severe cases^[2]. Metformin is the most widely used anti-diabetic drug of choice for diabetes^[3]. Recently, studies have shown that in addition to its hypoglycemic effect, it can reduce excessive endoplasmic reticulum stress, oxidative stress, and cell apoptosis, thereby exerting a protective effect on the kidneys^[4-6].

The 8-OHdG and 8-iso-PG are the biomarkers of DNA peroxidation^[7] and lipid peroxidation^[8] respectively. The excretion of 8-OHdG and 8-iso-PG in urine reflects the state of oxidative stress in the body index. However, the ability of metformin to improve 8-OHdG and 8-iso-PG in diabetic kidney disease has rarely been published. In this study, the renal protective effect of metformin on a model of type 2 diabetes mice and its effect on 8-OHdG and 8-iso-PG excretion in urine was observed, and the possible mechanism of reno-protective was explored.

Materials And Methods

1. animals

Eight-week-old SPF C57BL / 6J mice weighing 20-23 g, were purchased from Sandscobes Biotechnology Co., Ltd. All the mice were housed in 12h light/dark alternating regular illumination, ambient temperature (20 ± 1) °C, the humidity was (48 ± 10) % and free access to food and water. During the experiment period, animals were treated strictly

according to the rules in the Ethics Committee of the first affiliated hospital of the University of Science and Technology of China (Anhui Provincial Hospital). Every effort was made to minimize the number of animals used and their suffering.

2. experimental Grouping

After 7 days of acclimatization, mice were randomly divided into normal control group (n=8, fed with an ordinary diet until the end of the experiment) and high-fat diet (HFD) group (n=30, fed with HFD (containing 20 % protein, 20 % carbohydrate, 60 % fat until the end of the experiment)). After 16 weeks of feeding on a regular and high-fat diet, HFD mice had received a small dose of STZ (50 mg/kg was dissolved in 0.1mol/L citrate buffer, pH=4.2 and 3 days), and the NC group was injected with an equal amount of citrate buffer, and after a week, random blood glucose $\geq 16.7\text{mmol/L}$ for T2DM. Then the T2DM model mice (n=22) were randomly divided into 3 groups as follows: (1) T2DM group (n=7), in which the mice were received by gavage of the equivalent volume of normal saline for 8 weeks; (2) MET group (n = 8), in which the mice were received by gavage of Metformin (Shanghai shimeigun Pharmaceutical Company) at a dose of 250 mg/kg/day for 8 weeks. (3) GLIB group (n = 7), in which the mice were received by gavage of Glibenclamide (Tianjin Pacific Pharmaceutical Co., Ltd) at a dose of 2.5 mg/kg/day for 8 weeks. The mice in the normal control group were received a subcutaneous injection of the equivalent volume of normal saline for 8 weeks. After 8 weeks, the mice were weighed, fasting blood glucose was measured. Furthermore, blood, urine, and kidney tissue specimens were collected for further examination. The tissue specimens were stored at -80°C before further use.

3. Detection of biochemical indicators

Before the mice were sacrificed, they were placed in a metabolic cage to collect urine. Samples were used to assay for Alb, RBP, PCX, 8-OHdG, and 8-iso-PG by ELISA method and were tested for urine creatinine (UCR) by the picric acid method. Urinary Alb/UCR (UACR), urinary RBP /UCR (URCR), urinary PCX /UCR (UPCR), urinary 8-OHdG/UCR (UOHCR), and urinary 8-iso-PG/UCR (UISOCR) were calculated.

4. Electric microscope observation

The renal cortex was harvested and fixed with 2.5% glutaraldehyde to make ultrathin sections. Five visual fields of sections were examined in a JEM-1230 transmission electron microscope. GBMT (glomerular basement membrane thickness) was measured at five sites randomly. The total length of fused foot processes / total length of the basement membrane was measured and recorded as FPCR (foot process fusion rate). All parameters were measured by the Image J image analysis system.

5. Statistical analysis

Statistical analysis was performed using the Graphpad and spss 26.0 software. Data were expressed as mean \pm standard deviation (means \pm SD) and comparisons between two groups were performed using Student's t-test. Multi-group comparisons were analyzed using a one-way ANOVA test. If data were shown normally distributed, pairwise comparisons were conducted by LSD test. If not, pairwise comparisons were conducted by the Dunnett T3 test. All P values were two-sided, and a value of P less than 0.05 was considered statistically significant.

Results

1. Comparison of body weight and hematuria in each group

The weight of mice and biochemical results were presented in Table 1. Elevated levels of FBG, HbA1c, UACR, UPCR, URCR, and reduced weight were detected in the T2DM groups compared with the NC group ($p<0.05$). Following eight weeks of treatment with MET and GLIB, weight levels increased and FBG, HbA1c, UACR, UPCR, URCR decreased significantly in the GLIB and MET groups ($p<0.05$). Furthermore, decreased levels of UACR, UPCR and URCR were detected in the MET group compared with the GLIB group ($p<0.05$), but the levels of FBG and HbA1c were not significant different.

Table 1
Comparison of body weight and biochemical indexes among four groups (means \pm SD)

Groups	Weight(g)	HbA1c(nmol/L)	FBG(mmol/L)	UACR(mg/g)	UPCR(ug/g)	URCR(ug/g)
NC=8	27.85 \pm 1.61	1621.17 \pm 149.92	4.11 \pm 0.43	1.75 \pm 0.55	0.04 \pm 0.02	0.07 \pm 0.03
T2DM=7	19.35 \pm 2.00 ^a	2828.72 \pm 154.21 ^a	11.70 \pm 2.40 ^a	14.05 \pm 2.99 ^a	0.34 \pm 0.09 ^a	0.70 \pm 0.19 ^a
GLIB=7	23.51 \pm 0.85 ^{ab}	1891.17 \pm 212.16 ^{ab}	6.36 \pm 1.39 ^{ab}	8.12 \pm 1.54 ^{ab}	0.19 \pm 0.02 ^{ab}	0.40 \pm 0.05 ^{ab}
MET=8	23.48 \pm 1.73 ^{ab}	1985.99 \pm 224.17 ^{ab}	6.88 \pm 0.75 ^{ab}	5.32 \pm 0.82 ^{abc}	0.12 \pm 0.02 ^{abc}	0.25 \pm 0.03 ^{abc}
^a $P<0.05$ vs. NC group; ^b $P<0.05$ vs. T2DM group; ^c $P<0.05$ vs. GLIB group.						

2. Kidney pathology changes

Electron microscopy results showed that the thickness of the basement membrane in the NC group was uniform and clear, and the foot processes were basically not fused. The basement membrane in the T2DM group was thickened, the structure was fuzzy, and the foot processes were obviously fused and detached. The degree of GBMT and FPFR in the MET group and the GLIB group were improved compared with the DM group, and the difference between the two groups was statistically significant ($p<0.05$) These results are shown in Table 2 and Figure 1.

Table 2
Comparison of GBMT and FPFR among four groups (means \pm SD)

Groups	GBMT(um)	FPFR(%)
NC	0.19 \pm 0.02	0.11 \pm 0.04
T2DM	0.39 \pm 0.06 ^a	0.47 \pm 0.02 ^a
GLIB	0.35 \pm 0.02 ^{ab}	0.41 \pm 0.03 ^{ab}
MET	0.23 \pm 0.02 ^c	0.22 \pm 0.01 ^{ac}
^a $P<0.05$ vs. NC group; ^b $P<0.05$ vs. T2DM group; ^c $P<0.05$ vs. GLIB group.		

3. Comparison of UOHCR and UISOCR in each group

As illustrated in Table III, compared with the NC group, UOHCR and UISOCR increased in each diabetes group ($p<0.05$). Compared with the T2DM group, UOHCR and UISOCR were significantly lower in the MET group and the GLIB group ($p<0.05$), and the MET group was lower than the GLIB group ($p<0.05$).

Table 3 Comparison of Oxidative stress index among four groups (means±SD)

Groups	UOHCR(μg/g)	UISOCR(μg/g)
NC=8	0.31±0.09	1.32±0.47
T2DM=7	2.93±0.46 ^a	10.41±2.08 ^a
GLIB=7	1.82±0.20 ^{ab}	6.77±1.06 ^{ab}
MET=8	1.10±0.20 ^{abc}	4.51±0.92 ^{abc}

^aP<0.05 vs. NC group; ^bP<0.05 vs. T2DM group; ^cP<0.05 vs. GLIB group.

4. Correlation analysis

Correlation analysis showed that UOHCR showed positive correlations with UACR, UPCR, URCR (r=0.975, 0.978, and 0.958. all P<0.01), and UISOCR showed positive correlations with UACR, UPCR, URCR (r=0.976, 0.973 and 0.940. all P<0.01).

Discussion

Diabetic kidney disease (DKD) is a common microvascular complication of diabetes, which occurs in approximately 30% of patients with type 1 diabetes mellitus (T1DM) and 50% of T2DM patients^[9]. DKD is often accompanied by thickening of the glomerular basement membrane, tubular interstitial fibrosis, tubular atrophy, and altered podocyte morphology^[10, 11]. It has been shown that metformin treatment improves the renal structure and dysfunction associated with DKD^[5, 12, 13]. Furthermore, it has been shown that metformin exerts its renal protective effect independently of hypoglycemic^[14, 15]. In the present study, after 8 weeks of intervention in type 2 diabetic mice, the excretion level of urinary albumin (an indicator of glomerular damage), retinol-binding protein (an indicator of proximal tubular function), and podocalyxin (an indicator of podocyte damage) were significantly increased in the T2DM groups compared with the NC group compared with the normal group. Moreover, compared with the normal group, GBMT thickened and FPCR increased in the T2DM group, suggesting that diabetic mice have obvious kidney damage in the state of hyperglycemia. After metformin and glibenclamide intervention, the above indicators were ameliorated at different degrees compared with T2DM mice. In addition, the metformin group was significantly better than the glibenclamide group under the condition of similar glycemic control, suggesting that metformin has relatively better renal protection, which may be partly independent of the hypoglycemic effect.

8-OHdG(a biomarker of DNA oxidative damage)^[16]and 8-iso-PG(a biomarker of lipid peroxidation)^[17]are that reflect the state of oxidative stress in the body^[18]. Cellular interference caused by oxidative stress can lead to different changes in biomolecules (including DNA), with the nuclear base most sensitive to oxidative stress being guanine, whose damage results in a modified 8-OHdG^[19]. 8-iso-PG is generated by binding to arachidonic acid through reactive oxygen species-mediated lipid peroxidation^[20]. Urinary 8-OHdG and 8-iso-PG excretion can be used as indicators reflecting the oxidative stress status in vivo^[16, 21]. The observations in this study showed urinary 8-OHdG and 8-iso-PG were increased in type 2 diabetic mice. In addition, the excretion level of urinary 8-OHdG and 8-iso-PG were positively associated with UACR, UPCR, and URCR. This finding may partially suggest that oxidative stress is involved in the development of diabetic kidney disease.

Metformin is a basal antidiabetic drug for type 2 diabetes, and many basic and clinical studies have shown a better renal protective effect in recent years, and its mechanism may be multi-channel (AMPK/mTOR signaling pathway^[22], ER stress^[23], Autophagy^[24], Oxidative stress^[25]et al), in which oxidative stress plays a key role in the development and

progression of diabetic kidney disease (DKD)^[26]. It was shown that metformin can reduce DNA damage related to oxidative stress^[27] and lipid peroxidation^[28]. The study by Qi et al^[29] showed that in the ICH model of rats, the levels of lipid peroxidation antioxidant enzymes and 8-iso-PGF2 α were detected to assess oxidative stress. They found that metformin reduced oxidative stress and was maintained under ICH conditions^[30]. The present study showed after 8 weeks of intervention with metformin and glibenclamide, urine UOHCR and UISOCR in type 2 diabetic mice were significantly reduced, and the metformin group was lower than that of the glimepiride group, suggesting that metformin was more effective in reducing the risk of diabetic mice. Metformin reduces the oxidative stress mechanism is not yet clear. The study by Shambhoo S et al.^[31] found that metformin can improve the redox imbalance of rat red blood cells induced by rotenone, increase the activity of AMP-activated protein kinase and increase the antioxidant effect^[32]. Yang L et al. reported that metformin can exert an antioxidant effect through activation of the AMPK/Nrf2 pathway^[33]. In addition, metformin can also reduce the production of ROS by activating the AMPK pathway and inhibiting NADPH oxidase^[34].

Conclusion

In conclusion, metformin intervention has a clear protective effect on the kidney of type 2 diabetic mice, and the mechanism may be associated with its partial reduction of enhanced oxidative stress in the diabetic high glucose state in the body, and the complete mechanism needs to be further explored.

Abbreviations

MET: Metformin; GLIB: Glibenclamide; T2DM: Type 2 Diabetes Mellitus; STZ: streptozotocin; FBG: Fasting blood sugar; HbA1c: HemoglobinA1c; Cr: creatinine; Alb: Urine albumin; UACR: Urinary albumin/creatinine; PCX: Podocalyxin; UPCR: PCX/creatinine; RBP: Retinol-binding protein; GBMT: Glomerular basement membrane thickness; FPCR: Foot process fusion rate; 8-OHdG: 8-Hydroxy-2'-Deoxyguanosine; 8-iso-PG: 8-iso-prostaglandin. DKD: Diabetic kidney disease.

Declarations

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Authors' contributions

Ye Shandong: Conceptualization. Wen Wenjie, Zhang Qilun, Bi shuangjie, Xue Jingfan, Wu Xiaoying: animal assay (equal). Zhou Wan, Wang Wei: Software and Visualization. Wen Wenjie: Writing original draft.

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Availability of data and materials

All data generated or analyzed during this study and supporting our findings are included and can be found in the manuscript. The raw data can be provided by corresponding author on reasonable request.

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations. The animal experimental procedures were prepared following the ARRIVE guidelines. The protocol of the experiments for animal use was approved by the Ethics Committee of the first affiliated hospital of the University of Science and Technology of China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

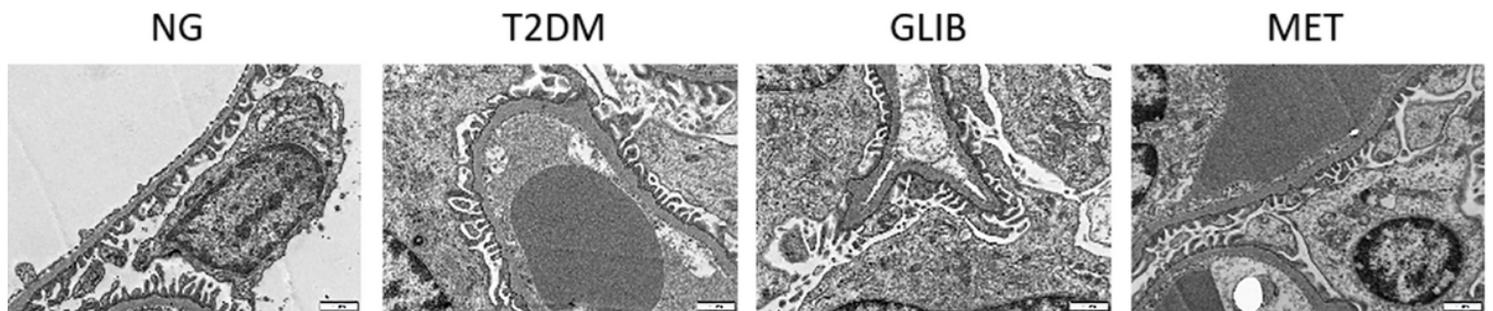


Figure 1

The histopathological changes in mice under electron microscope among four groups(20000×)