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Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through activation of AMPK signaling pathway

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ABSTRACT

Background: Nephropathy is the main problem of diabetes and can be classified into several phases according to the presence of albuminuria. Adenosine monophosphate-activated protein kinase (AMPK) operates as a sensor of energy charge.
Objectives: The aim of our study was to evaluate the reno-protective properties of AMPK signaling pathway against streptozotocin (STZ)-induced nephropathy in the rat.
Materials and Methods: Forty male Wistar rats were randomly distributed into four groups. Group 1 was normal rats (N group); group 2 was diabetic rats (D group); group 3 received diabetic rats + metformin (DM group), and group 4 received giabetic rats + metformin + dorsomorphin (DMD group). Serum albumin, uric acid, total protein and creatinine for estimation of renal injury were measured. Finally, the histological study was evaluated.
Results: Reduction of body weight, albumin and total protein in the diabetic rat was reversed by metformin administration. Our results showed that serum uric acid and creatinine were significantly increased in diabetic rats and decreased after treatment with metformin in diabetic rats. AMPK improved the histopathology and morphological changes in STZ-induced diabetic rats. Administration of dorsomorphin (AMPK inhibitor) with metformin can reverse the beneficial effects of AMPK.
Conclusions: AMPK signaling pathway ameliorates diabetic nephropathy by modifications of serum albumin, uric acid, total protein, creatinine and attenuation of kidney damage.

Original Article

Implication for health policy/practice/research/medical education:
In this experimental study, we found that activation of AMPK via metformin can protect nephropathy against STZ-induced diabetes in models of rats. The main mechanism of AMPK in renoprotective effects was increased serum albumin and total protein levels and decreased serum uric acid and creatinine levels after treatment with metformin in diabetic rats.
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1. Background

Nephropathy is the main complication of diabetes mellitus and show one of the main challenges for modern nephrology as the most cause of chronic kidney disease, accounting for common about 30%-40% of new cases of diabetes (1). Diabetic nephropathy, also called diabetic kidney disease, can be classified into several phases conforming to the presence of albuminuria and the degree of chronic kidney disease (2). In the kidneys, glucose by binding to proteins, lead to the production of advanced glycosylation end products (AGEs). AGE contribute to renal damage by stimulation and release of proinflammatory cytokines and expression of growth factors in the development of diabetic nephropathy (3). A diabetes preventive role for medications such as metformin has been suggested (4). Metformin is an anti-diabetic medicine used for in the treatment of diabetes mellitus (type II), and it is used as the treatment for other metabolic diseases (5,6). The mechanism of action of metformin is suggested to be through activation of adenosine monophosphate-activated protein kinase (AMPK) (7,8). The main objective of metformin seems to be the mitochondrial respiratory chain complex I leading to activation of AMPK (9). AMPK operates as a sensor of energy charge (10) that is activated with elevation of AMP (11) concomitant with decreased cellular ATP levels (12).

2. Objectives

The present study aimed to evaluate the nephroprotective effects of AMPK signaling pathway in rodent models of streptozotocin-induced diabetic nephropathy.

3. Materials and Methods

3.1. Materials

Materials are rats (prepared by the faculty of medical school, Tehran University of Medical Sciences), streptozotocin (Sigma-Aldrich, USA), metformin and dorsomorphin dihydrochloride (Tocris Bioscience, USA)

3.2. Methods

3.2.1. Animals

Forty male Wistar rats weighing 210±10 g, in the department of pharmacology, school of medicine, Tehran Medical Science University, were used in this study. The animals were in a room with a temperature of 22 ± 2°C and 12-h light/12-h dark cycle and free access to food and water. The rats were weighed and were kept for 20 to 24 hours are hungry and randomly allocated into four groups as follows:

Group I (N group); normal rats

Group II (D group); diabetic rats

Group III (DM group); diabetic rats + metformin

Group IV (DMD group); diabetic rats + metformin + dorsomorphin.

Metformin activates AMPK can be administered orally (300 mg/kg) in the body and dorsomorphin (0.2 mg/kg) daily intraperitoneal injection (IP) injection was used as an inhibitor of the activity of AMPK. Diabetes was induced in rats of groups II, III and IV were injected with single intraperitoneal of 65 mg/kg of streptozotocin (STZ). STZ was freshly dissolved in 0.05 M citrate buffer. Blood glucose levels were measured after 48 hours with blood from the tail vein and animals whose blood glucose with 400 mg/dL were considered as diabetic rats were used in the study. In the groups which received dorsomorphin and metformin, drug used 72 hours after induction of diabetes, and as each day until 6 weeks after induction of diabetes. Evaluation of the effect of serum parameters amd histopatologic study were done on 6 weeks after induction of diabetes. Body weight of each animal was determined at the initiation and end of the study.

3.2.2. Serum parameters

At the end of the treatment period, rats were anesthetized with pentobarbital intended (70 mg/kg, IP), blood samples were collected into tubes without anticoagulant and centrifuged at 3000 rpm for 10 minutes to sepatation of serum, and the serum was stored at -70°C untile assay. Serum samples were used for measurement of albumin, uric acid, total protein and creatinine by enzymatic colorimetric methods according to the standard protocol of manufacturer's instructions (13).

3.2.3. Histological study

For renal morphological studies, in 45th after induced nephropathy, the right kidney was isolated and fixed in buffered formalin, dehydrated in histoligical paraffin. Renal sample was sectioned (5 μm) and stained with hematoxylin and eosin (13).

3.3. Ethical issues

The research followed the tenets of the Declaration of Helsinki. This project was approved by Ethics Committee of Tehran University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Tehran University of Medical Sciences.

3.4. Statistical analysis

One-way analysis of variance (ANOVA) analysis followed by Tukey post hoc test was used for evaluation. All data are presented as means ± standard deviation (SD). Results were considered significant when P value <0.05.

Table 1. Assessment of body weight

	N	D	DM	DMD
Body weight (g, 6 weeks)	274.7±5.08	162.5±3.39***	171.2±4.19#	166.1±3.48

*** $P<0.001$ compared to normal group; # $P<0.05$ compared to diabetic group.
N: Normal group (Non-diabetic group), D: Diabetic group, DM: Diabetic + Metformin group and DMD: Diabetic + Metformin + Dorsomorphin group.

4. Results

4.1. Body weight

Table 1 demonstrates the effect of metformin on body weight. Diabetic rats (D group) show exhibited significant weight loss period compared to the control rats. At the end of 6 weeks treatment, the body weight of animals treated with metformin (300 mg/kg) was significantly improved compared with the STZ-diabetic group ($P<0.05$). However, treatment with metformin and dorsomorphin cannot be attenuated the body weight in the DMD group.

4.2. Serum albumin assessment

Administration of STZ led to significant decrease in serum albumin levels in the D group as compared to the normal group (N group) throughout 6 weeks after induction of diabetes by STZ in rats ($P<0.001$). Furthermore, at the end of 6 weeks treatment, diabetic animals treated with 300 mg/kg metformin demonstrated attenuating in the serum albumin levels ($P<0.05$). In diabetic rats treated with metformin and dorsomorphin, a decrease in the serum albumin levels was detected (Figure 1).

4.3. Serum uric acid measurement

The diabetic animals showed a significant increase in the level of uric acid compare to the normal group ($P<0.001$). After treatment with metformin in group III (DM group), serum uric acid decreased significantly ($P<0.001$). Also, the diabetic rats treated with metformin and dorsomorphin (DMD group) showed a significant reduction of serum uric acid levels as compared to the group II ($P<0.05$, Figure 2).

4.4. Assessment the level of total proteins

After 6 weeks, STZ-diabetic rats (group II) showed a significant decrease in the levels of total protein when compared with normal group (group 1) ($P<0.001$). Animal's treatment with 300 mg/kg metformin (group III) showed a significant increase in the levels of total proteins compared with their D group ($P<0.01$). Interestingly, in group DMD (group IV), concomitant metformin and dorsomorphin administration to inhibit AMPK signaling pathway, showed a significant increase in level of serum total proteins compared with diabetic control (group 2) after 6 weeks ($P<0.05$, Figure 3).

4.5. Assessment the level of serum creatinine

STZ-diabetic rats (group II) showed a significant increase in the serum levels of creatinine ($P<0.001$). Furthermore, the levels of creatinine significantly decreased in the serum of DM group (group III) compared to diabetic rats ($P<0.01$, Figure 4). Also, this parameter was not significant in diabetic rats treated with metformin and dorsomorphin (DMD group).

4.6. Histological study

Figure 5 shows the histological study of renal tissues of rats from the non-diabetic group and experimental

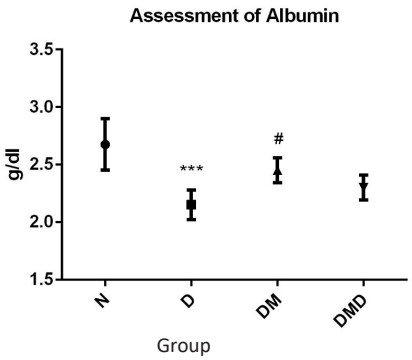

Figure 1. Assessment of Albumin. *** $P<0.001$ compared to Normal group, # $P<0.05$ compared to Diabetic group.
N: Normal group (non-diabetic group), D: Diabetic group, DM: Diabetic + Metformin group and DMD: Diabetic + Metformin + Dorsomorphin group.

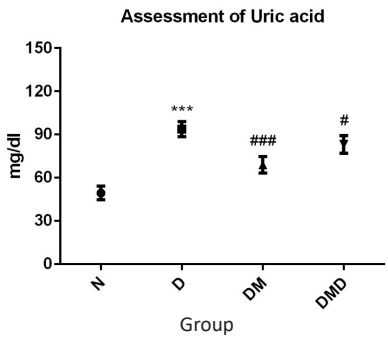

Figure 2. Assessment of Uric acid. *** $P<0.001$ compared to normal group, # $P<0.05$ and ### $P<0.001$ compared to diabetic group.
N: Normal group (Non-diabetic group), D: Diabetic group, DM: Diabetic + Metformin group and DMD: Diabetic + Metformin + Dorsomorphin group.

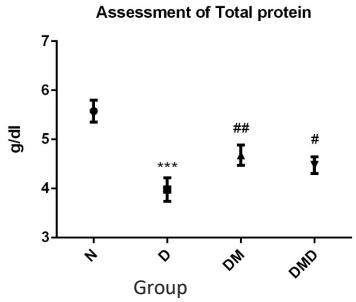

Figure 3. Assessment of Total Protein.
*** $P<0.001$ compared to normal group, # $P<0.05$ and ## $P<0.01$ compared to diabetic group.
N: Normal group (Non-diabetic group), D: Diabetic group, DM: Diabetic + Metformin group and DMD: Diabetic + Metformin + Dorsomorphin group.

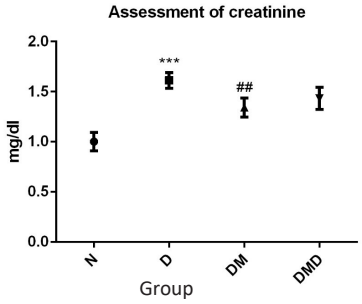

Figure 4. Assessment of serum creatinine.
*** $P<0.001$ compared to Normal group, ## $P<0.01$ to diabetic group.
N: Normal group (Non-diabetic group), D: Diabetic group, DM: Diabetic + Metformin group and DMD: Diabetic + Metformin + Dorsomorphin group.

groups. In the non-diabetic group, renal glomeruli seemed to be normal. In the diabetic group, increased tubular damage, tubulointerstitial injuries and glomerular damage. Metformin significantly improved the tubular damage in diabetic renal damage. However, dorsomorphin treatment with metformin reversed the beneficial effects of metformin on renal histopathological in diabetic rats.

5. Discussion

In this study, we evaluated the reno-protective effects of AMPK signaling pathway in the diabetic nephropathy induced by STZ in rat. Treatment with metformin in diabetic rat attenuated the body weight in compared to untreated diabetic rats ($P<0.05$). However, the results in this study showed that administration metformin with dorsomorphin did not improve the body weight in diabetic rats. We showed that metformin, an AMPK activator, significantly increased the albumin ($P<0.05$) and total protein ($P<0.01$) and also, decreased the levels of creatinine ($P<0.01$) and uric acid ($P<0.001$) in the

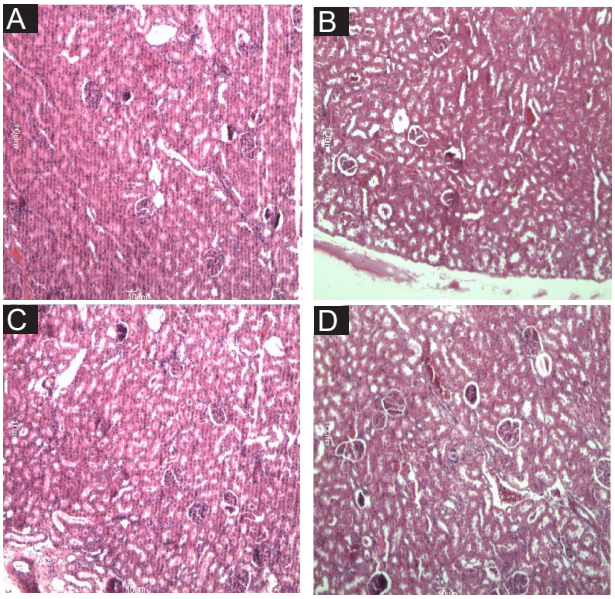

Figure 5. Assessment of histological study. A: Normal group (Non-diabetic group), B: Diabetic group, C: Diabetic + Metformin group and D: Diabetic + Metformin + Dorsomorphin group.

serum of diabetic rats. In addition, co-treatment of dorsomorphin with metformin decreased the positive effects of metformin on serum albumin and creatinine. Furthermore, the evaluation of histopathological studies demonstrated that activate AMPK signaling pathway attenuate the kidney tissue damage that is caused by STZ-diabetes.

STZ-induced type 1 diabetic rats are associated with severe decrease of body weight caused by hyperglycemia, loss of tissue proteins (14), muscular tissue and adipose tissue (15). Additionally, the increase in the levels of serum uric acid and creatinine (16) and the decrease in the levels of serum albumin and total protein (17) have been associated with diabetic kidney disease. STZ induced diabetes (type 1) has been determined as a useful experimental model for study of diabetes because STZ causes selective destruction of the β-cell in pancreatic islets (18, 19). It has been shown that dorsomorphin (AMPK inhibitor) can reverse the beneficial effects of AMPK (20). Metformin is currently the drug of choice for the management of diabetes. It improves plasma levels of glucagon-like peptide 1 (GLP-1), peripheral and liver sensitivity to insulin, decreases hepatic glucose production, and causes weight reduction (21). A recent study demonstrated that metformin stimulates the adenosine monophosphate protein kinase signaling pathway (22). Lee et al reported that phosphorylation of AMPK signaling levels is diminished in the diabetes-induced renal hypertrophy (23). In addition, Kim et al suggested that AMPK activation in renal tissues by metformin attenuated, loss of podocyte in nephropathy

of diabetes (24). Furthermore, it has been shown that treatment with metformin significantly decreased albuminuria in diabetic patients (25) and serum creatinine in STZ-nicotinamide-induced diabetic nephropathy in rats (26) and also serum uric acid in gout patients (27). Renal cell damage induced by cisplatin-induced tubular cell apoptosis and acute kidney injury attenuated by activation of AMPK with metformin (28). In another study, in mouse renal fibroblasts activation of AMPK pathway using metformin diminished the renal interstitial fibrosis in chronic kidney disease (29). Zhang et al showed that the expression of phosphorylated AMPK via metformin attenuated metabolic renal function in mice (30).

6. Conclusions

The data of this study suggested that AMPK signaling pathway has a protective effect on kidney in the diabetic nephropathy induced by STZ in the rat. Our results suggested that AMPK showed protective effect by attenuating complications of diabetic nephropathy through preventing a rise in uric acid and creatinine levels, and reduced albumin and total protein levels.

Authors' contribution

AH, HA, SJ, MH, SE and AD provided technical assistance, collection and preparation of the manuscript. AA and SMT analyzed the pathology data. SE and AD designed, supervised the study and prepared the final draft of the article.

Conflicts of interest

The authors declared no competing interests.

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