Metformin exerts renoprotective effect by reducing proteinuria in spontaneously-hypertensive rats

Yuanyuan Liu*, Chengjian Wang, Enyuan Zhang
Department of Cardiology, Tianjin Chest Hospital, Tianjin, PR China

*For correspondence: Email: uikb2c@163.com

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Abstract

Purpose: To study the mechanism involved in metformin (DMBG)-induced reduction of albuminuria in spontaneously-hypertensive rats (SHRs).

Methods: The SHRs were divided into DMBG SHR group (n = 30) given DMBG (300 mg/kg) and NC SHR group (n = 10) given distilled water (2 ml/day), while Wistar-Kyoto rats (WKY) rats were divided into DMBG WKY group (n = 30) given DMBG (300 mg/kg ) and NC WKY group (n = 10) administered distilled water (2 ml/day). Urinary protein levels were measured and compared. Inflammatory infiltration in renal tissue, and expression of vascular endothelial growth factor (VEGF) were assessed. Abdominal aortic blood was used to measure serum levels of creatinine and blood urea nitrogen (BUN).

Results: Urinary protein was markedly elevated in SHRs, while BUN in SHRs was lowered, relative to WKY rats (p < 0.05). Moreover, inflammatory cell population was lower in DMBG SHRs group than in WKY rats (p < 0.05). Kidney expressions of VEGF in SHR and WKY rats treated with metformin were significantly reduced, relative to those in untreated rats, but VEGF-A in DMBG SHR rats was markedly elevated, relative to NC SHR group, and was also raised in DMBG WKY, when compared with NC WKY rats (p < 0.05).

Conclusion: Metformin reduces albuminemia in spontaneously hypertensive rats by up-regulating VEGF expression, thereby exerting renoprotective effect. This finding provides ideas for research and development of other therapeutic approaches.

Keywords: Metformin, Spontaneous hypertension, Renoprotective, Albuminuria, Kidney, Vascular endothelial growth factor (VEGF)

INTRODUCTION

Hypertension refers to a clinical syndrome in which systemic arterial blood pressure is increased with or without functional or organic damage to the heart, brain, kidney or any other organ [1]. Arising from the development of the Chinese economy and improvement in living standard, the incidence of hypertension is on the increase. Hypertension often damages many organs, among which the kidney occupies a prominent position [2]. Studies have shown that the probability of renal injury in patients with hypertension is higher than that in normal people, and the incidence of chronic renal insufficiency increases year by year [3].
early clinical symptoms of hypertension are few, and the disease is of slow onset, thereby making it difficult for the affected persons to detect it early. However, when hypertension-associated renal injury symptom such as albuminuria appears, the quality of life of the patient is adversely affected [4]. Therefore, timely control of hypertension and inhibition of renal injury in hypertensive patients have always been the focus of medical research on hypertension worldwide. Metformin (DMBG) is a biguanide drug which inhibits glucose transport, increases insulin sensitivity, reduces blood glucose, and minimizes diabetic complications by reducing blood lipids and increasing promyelocytic activity [5]. It has been reported that metformin reduced urinary protein level in patients with severe hypertension, but the related mechanism is not yet known [6]. This investigation was carried out to unravel the effect of (DMBG) on proteinuria in spontaneously hypertensive rats, and the mechanism involved.

EXPERIMENTAL

Animals

A total of 40 SHRs and 40 WKY rats aged 6.5 - 7.0 weeks, with mean body weight of 122.24 ± 20.42 g, were bought from Beijing tong Lihua Experimental Co. Ltd (biological license: experimental animals SCXK20120001).

This research was approved by the Animal Ethical Committee of Tianjin Chest Hospital, Taierzhuang South Road, Jinnan District, Tianjin City, China (approval no. 201935578), and was carried out in line with the "Principles of Laboratory Animal Care" (NIH, 1985) [7].

Treatment

The rats were kept at room temperature of 22 - 25 °C, relative humidity of 40 - 60 %, and noise level of ≤ 45dB, in an environment with 12-h round-the-clock natural light, and fed with standard feed and distilled water. The SHR rats were divided into DMBG SHR group (n = 30, 300 mg/kg) and NC SHR group (n = 10), while the WKY rats were assigned to DMBG WKY group (n = 30) and NC WKY group (n = 10). The NC SHR group and NC WKY group were given drinking water for 5 months.

Evaluation of treatment indicators

Blood glucose

Following a 12-h fast, blood was taken from the tail artery of each rat and subjected to centrifugation at 3000 rpm for 15 min. The serum was kept in a refrigerator at -80 °C to avoid repeated freezing and thawing, and blood glucose was measured using automatic analyzer.

Systolic blood pressure

The rats were placed in a quiet environment, and their blood pressure was determined using tail cuff technique. Mean systolic blood pressure (SBP) was obtained three times within 10 min.

Urinary protein

The urine of the rats was collected for 24 h, and the total urine volume was recorded. The urine was spun at 3000 rpm for 15 min, and its protein content was determined using an Automatic analyzer.

The animals were anesthetized, and 5 mL of abdominal aortic blood was taken from each rat. The blood was centrifugated at 3000g at room temperature for 15 min. Serum creatinine (Cre) and BUN levels of the rats were determined using Automatic analyzer. Tissue was taken from the upper pole of the left kidney, and processed for light microscopy. Paraffinized sections of the kidney tissues were subjected to H & E staining and examined under the microscope for presence of inflammatory infiltration. The expression of VEGF in the kidney was assayed using immunohistochemistry. The excised rat kidney tissue was made into a homogenate which was stored in polypropylene tube, and the VEGF-A protein concentration was determined with enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

All measurement data are expressed as mean ± standard deviation (SD). Inter-group comparison was done using t-test. Counting data are expressed as percentage, and t-test was used for comparison between groups. Grade data were compared using Ridit test. All statistical analyses were done using SPSS 20.0 software package. Statistical significance was assumed at p < 0.05.

RESULTS

Blood glucose, SBP and body weight

At the end of treatment, there were no significant differences in blood glucose and body weight between SHR rats and WKY rats (p > 0.05). The systolic blood pressure of rats treated with
metformin was significantly different from that of untreated rats ($p < 0.05$, Table 1).

**Urinary protein, Cre and BUN levels of rats at late stage of treatment**

Rat urine protein content in DMBG SHR group was significantly higher than that of DMBG WKY group, and BUN level of DMBG SHR rats was significantly higher than that of DMBG WKY group. The urine protein and Cre levels in the metformin-treated rats were significantly lower than the corresponding levels in untreated rats ($p < 0.05$). However, metformin did not exert any marked effect on BUN levels in SHR rats and DMBG rats ($p > 0.05$). These data are shown in Table 2.

**Levels of inflammatory cells in rat kidneys at late stage of treatment**

Histological examination showed that NC WKY group and DMBG WKY group had no significant morphological abnormalities in renal tissue, while rats in DMBG SHR group had significantly lower inflammatory cell infiltration than rats in the NC SHR group. Quantitative analysis of H & E stained sections showed that the level of inflammatory cells in the liver of rats was markedly elevated in SHRs, relative to that in the liver of the WKY group ($p < 0.05$). Moreover, there were significantly higher number of inflammatory cells in DMBG SHR group than in WKY group ($p < 0.05$; Table 3 and Figure 1).

**Renal VEGF expression**

Immunohistochemical studies showed that renal VEGF expressions in SHR rats and WKY rats treated with metformin were downregulated, relative to WKY and SHR rats without metformin treatment.

**Table 3: Levels of inflammatory cells in rat kidney at late stage of treatment**

| Group | n   | Inflammatory cells (cells/HPF) |
|-------|-----|---------------------------------|
| SHR   | 30  | 153.18 ± 20.86                 |
| NC SHR| 10  | 286.16 ± 26.18a                |
| DMBG WKY | 30  | 123.49 ± 19.48ab               |
| NC WKY| 10  | 134.72 ± 18.46ab               |

$^{a}p < 0.05$, vs DMBG SHR group; $^{b}p < 0.05$, vs NC SHR group. Values are expressed as mean ± SD

**Figure 1:** Rat kidney pathology of the various groups. A: renal tissue of rat from NC WKY group; B: kidney tissue of rat from DMBG WKY group; C: renal tissue of rat from NC SHR group; D: kidney tissue of rat from DMBG SHR group (H & E)

**Table 1: Systolic blood pressure, blood glucose and body weight**

| Group | n   | SBP (mmHg) | Blood glucose (mmol/L) | Body weight (g) |
|-------|-----|------------|------------------------|-----------------|
| SHR   | 30  | 204.18 ± 7.14 | 7.16 ± 0.15          | 358.16 ± 18.16  |
| NC SHR| 10  | 208.46 ± 5.16 | 7.13 ± 0.45          | 363.15 ± 17.49  |
| DMBG WKY | 30  | 122.42 ± 5.13 | 6.93 ± 0.41          | 355.18 ± 18.12  |
| NC WKY| 10  | 123.48 ± 3.46 | 7.06 ± 0.23          | 362.15 ± 20.44  |

Values are expressed as mean ± SD

**Table 2: Urinary protein, Cre and BUN levels at the late stage of treatment**

| Group | n   | Urinary protein (mg/24h) | Cre (μmol/L) | BUN (mmol/L) |
|-------|-----|--------------------------|--------------|--------------|
| SHR   | 30  | 28.16 ± 7.46             | 40.15 ± 1.46 | 10.43 ± 0.16 |
| NC SHR| 10  | 47.76 ± 10.46a           | 51.43 ± 1.86a| 11.43 ± 1.26 |
| DMBG WKY | 30  | 16.45 ± 3.18ab           | 41.16 ± 1.41 | 8.16 ± 0.73ab|
| NC WKY| 10  | 19.73 ± 3.52abc         | 49.15 ± 1.67c| 8.49 ± 0.82abc|

Data are mean ± SD, $^{a}p < 0.05$, vs DMBG SHR group; $^{b}p < 0.05$, vs DMBG SHR group; $^{c}p < 0.05$, vs DMBG WKY group
As shown in Table 4 and Figure 2, ELISA revealed that VEGF-A level in the DMBG SHR group was significantly higher than that in the NC SHR group, and VEGF-A level in the DMBG WKY group was significantly higher than that in the NC WKY group (p < 0.05).

Table 4: Renal VEGF expression among the groups at the late stage of treatment

| Group     | n   | VEGF-A (pg/mg) |
|-----------|-----|---------------|
| SHR       | 30  | 175.42 ± 20.15|
| DMBG SHR  | 30  | 175.42 ± 20.15|
| NC SHR    | 10  | 69.51 ± 11.15a|
| DMBG WKY  | 30  | 152.58 ± 14.20|
| NC WKY    | 10  | 68.25 ± 8.15b |

\( a p < 0.05, \) vs DMBR SHR group; \( b p < 0.05, \) vs NC SHR. Values are expressed as mean ± SD

Creatinine (Cre) is a product of muscle metabolism which is filtered out of the body through glomerular filtration [11]. Under normal circumstances, the daily production of Cre remains almost constant, and the concentration of Cre is relatively stable. Thus, Cre is clinically used as an indicator of glomerular function [12]. In this study, the urine protein and Cre levels of metformin-treated rats were significantly lower than those of untreated rats, suggesting that metformin enhanced glomerular filtration and reduced the level of proteinuria in hypertensive rats to a certain extent. This indicates that metformin exerts renoprotective effect, which is similar to the experimental results of Yeqiong [13].

Vascular endothelial growth factor (VEGF) is a highly specific growth factor in vascular endothelial cells. It increases vascular permeability, promotes formation of new capillaries, improves the activity of plasma-activating factor, and enhances the migration and proliferation of vascular endothelial cells [14]. Recent studies have shown that VEGF-A enhances and maintains glomerular filtration function which, when impaired, results in proteinuria [15]. In this study, the immunohistochemical status after metformin treatment of SHR and WKY rats revealed that renal expression of VEGF was significantly increased, when compared with VEGF-A expression in untreated rats. In DMBG SHR rats, VEGF-A level was markedly higher than that in NC SHR group, and VEGF-A level of rats in DMBG WKY group was elevated, relative to NC WKY group. This suggests that metformin improves glomerular filtration and reduces proteinuria by upregulating VEGF levels.

CONCLUSION

Metformin reduces proteinuria in spontaneously hypertensive rats and exerts renoprotective
effect through the upregulation of VEGF. These results provide a new direction for studying the mechanism involved in the protection of the kidney by metformin.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Yuanyuan Liu conceived and designed the study, Yuanyuan Liu, Chengjian Wang, Enyuan Zhang collected and analyzed the data. Yuanyuan Liu wrote the manuscript.

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