

# POLY(ADP-RIBOSE) POLYMERASE INHIBITOR COUNTERACTS ALBUMINURIA IN DIABETIC RATS

Albuminuria, protein albumin present in one's urine, is indicative of kidney dysfunction and is one of the markers of kidney disease in patients with diabetes mellitus. Evidence for the key role of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) in chronic diabetic complications in the heart, retina, and peripheral nerves is emerging.

This study investigated the effects of the PARP inhibitor 1,5-isoquinolinediol (ISO) on albuminuria in rats that have been made diabetic with streptozotocin ( $N=31$ ). ISO (3 mg/kg body weight<sup>-1</sup> d<sup>-1</sup>, intraperitoneally) was administered for 10 weeks after two initial weeks without treatment. ISO did not have any effect on weight gain or blood glucose concentrations in either non-diabetic or diabetic rats. At the end of the study, body weights were similarly reduced and blood glucose concentrations similarly increased in untreated and ISO-treated diabetic rats compared with non-diabetic controls. However, the ISO-treated diabetic group showed a significant decrease in consumed water and urination compared with untreated diabetic group ( $P<.01$ ). Most importantly, ISO treatment reduced urinary albumin excretion to the levels present in non-diabetic rats ( $P<.01$  compared with untreated diabetic group).

In conclusion, our study indicates that PARP inhibition is associated with a beneficial effect on urinary albumin excretion, a key marker of diabetic kidney disease. Our findings suggest an important role for PARP in diabetic nephropathy. Studies of other markers of diabetic kidney disease in the streptozotocin-diabetic rat model are in progress.

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## INTRODUCTION

Diabetes is a disease characterized by high blood glucose levels above the normal range (eg, 4–7 mmol/L in human blood). People with diabetes either do not produce enough insulin for the body or the insulin that is made is not potent enough for the body, thus leading to increased sugar levels in the blood.

Albuminuria is the condition when an excess amount of albumin is present in one's urine. This symptom occurs frequently in diabetic patients. Albumin in blood helps with the regulation of the osmotic pressure of blood. Albuminuria indicates damaged kidneys that are allowing proteins to slip out of the kidney's filtration system.

Poly(ADP-ribose) polymerase (PARP) inhibitors have been reported to be able to prevent and alleviate diabetic complications by multiple mechanisms. One of PARP inhibitors, 1, 5- isoquinolinediol (ISO), has been applied to treat diabetic neuropathy. This study investigated the effect of ISO on albuminuria in diabetic rats and showed the possibilities of application of ISO in diabetic kidney malfunction treatment.

## METHODS

### Body weight and blood glucose

At the beginning and at the end of treatment (10 weeks), body weight (g) and glucose level of blood taken from tail vein were checked. Water consumption. Rats divided into four experimental groups (C=control, C+I=ISO-treated control, D=diabetic, D+I=ISO-treated diabetic,  $n=5$  to 12) were placed separately into metabolic cages for 24 hours (Table 1). One hundred mL water bottles were placed into the inserts on each cage. The rats were given 24 hours to consume as much water as they wanted. The water bottles were checked and refilled every 8 hours throughout the 24-hour period to see how much water each rat had consumed.

### Urination

During this 24-hour period, a 15 mL (or 50 mL in the case of diabetic rats) centrifuge tube was connected to a screen mesh outlet on the bottom of a metabolic cage. Rat urine was collected from these tubes after 24 hours.

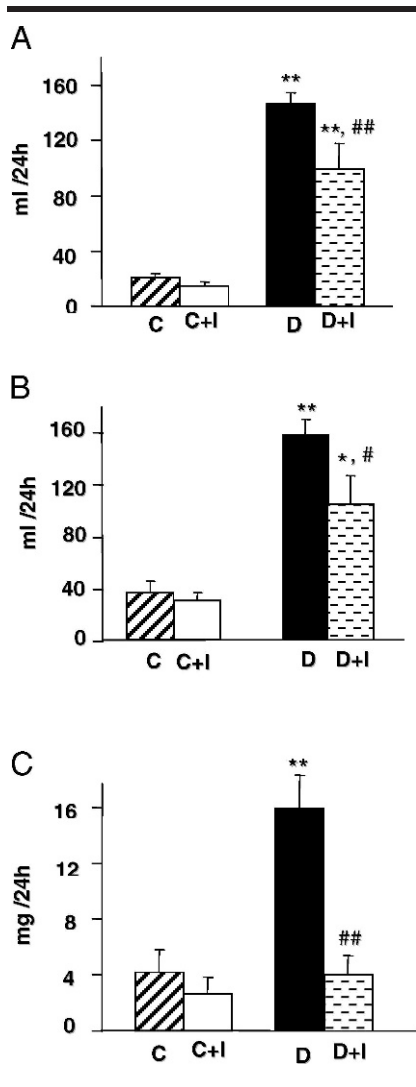
**Table 1. Weight gain (g) and blood glucose level (mmol/L)**

	C	C+ISO*	D	D+ISO
Initial body weight, g	290.5±2.3	295.9±3.2	287.8±3.8	298.1±3.3
Final body weight, g	565.0±21.7	537.4±16.0	353.2±13.0**	343.1±14.1**
Initial blood glucose, mmol/L	5.7±0.2	6.0±0.2	25.4±1.2**	26.6±0.9**
Final blood glucose, mmol/L	5.5±0.4	5.2±0.2	26.1±1.3**	25.0±0.9**

\* ISO=1, 5-isoquinolinediol

\*\*  $P<.01$  vs control group ( $n=5-12$ )

C=control, C+I=ISO treated control, D=diabetic, D+I=ISO treated diabetic



**Fig. 1.** Consumed water (A), urination volume (B), urine albumin excretion (C) in control and diabetic rats treated with or without ISO. C=control, C+I=ISO treated control, D=diabetic, D+I= ISO treated diabetic. \*\* $P < .01$  vs control group; #  $P < .05$  vs untreated diabetic group ( $n=5-12$ )

### Albumin assay

1 mL of urine was placed in a smaller centrifuge tube. Each tube was then centrifuged at 1000 rpm for 5 minutes. After centrifugation, the supernatant was collected from the tubes. These samples were then frozen at  $-20^{\circ}\text{C}$ . A week later, the samples were taken out of the freezer, thawed at room temperature, and centrifuged again. The supernatant was used for albumin assay. Measurement was carried out using a rat albumin ELISA (enzyme linked immuno sorbent assay) assay kit (Nephurat II, Exocell, Philadelphia, Penn). Using competitive ELISA method, urine albumin concentrations were obtained with triplicates.

## RESULTS

ISO did not have any effect on weight gain or blood glucose concentrations in either non-diabetic or diabetic rats (Table 1). At the end of the study, body weights were similarly reduced and blood glucose concentrations similarly increased in untreated and ISO-treated diabetic rats compared with non-diabetic controls ( $P < .01$ ). Figure 1A reflects the amount of water consumed by different experimental groups over the 24-hour period ( $n=5$  to 12). The diabetic group (D) consumed more water than the control (C) group ( $P < .01$ ). The ISO treated diabetic group (D+I) showed a significant decrease in consumed water compared to untreated diabetic group ( $P < .05$ ). There was no significant difference between the untreated and ISO treated control groups ( $P > .05$ ). Based on this data, we concluded that the agent ISO did reduce the amount of water consumed by the rats.

The 24-hour urination volume in the experimental groups is shown in

Figure 1B ( $n=5$  to 12). The urination volume was significantly higher in untreated diabetic group (D) than in non-diabetic controls ( $P < .01$ ). We found that ISO treatment of diabetic rats significantly reduced their urination ( $P < .01$ ). There was no significant difference between untreated and ISO treated control groups (C+I). Based on these results, we concluded that ISO treated diabetic rats urinated much less than untreated diabetic group ( $P < .01$ ).

Figure 1C illustrates the amount of albumin excreted in the rat's urine after the 24-hour period. The untreated diabetic group (D) showed significantly higher urinary albumin excretion compared with the control group ( $P < .01$ ). The ISO treated diabetic group did not significantly differ from the control group ( $P > .05$ ). ISO obviously lowered urinary albumin excretion in diabetic rats (D+I) ( $P < .01$ ).

## CONCLUSION

Whereas ISO did not have any effect on weight gain or blood glucose concentrations in either non-diabetic or diabetic rats, it significantly decreased diabetes-induced urinary albumin excretion. This is indicative of the beneficial effect of PARP inhibition on diabetic albuminuria, a key marker of diabetic kidney disease. Our findings are first to show an important role for PARP in diabetic nephropathy. Studies of other markers of diabetic kidney disease in the streptozotocin-diabetic rat model are in progress.

### ACKNOWLEDGMENT

This study was supported by NIH grant DK 010720 to Dr. Irina G. Obrosova.