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BRIEF COMMUNICATION

EFFECT OF IRBESARTAN ON STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY: AN INTERVENTIONARY STUDY

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ABSTRACT

Effect of irbesartan, an angiotensin II receptor antagonist, was studied in streptozotocin (STZ) induced diabetic nephropathy. Polyuria, proteinuria, blood urea, creatinine clearance, and urinary electrolytes were determined to assess kidney damage. There was a significant increase in urine volume, urinary protein and blood urea in STZ induced diabetic rats. On the other hand, irbesartan treatment resulted in a significant reduction in urinary protein and blood urea in these rats. Irbesartan treatment also improved creatinine clearance and exhibited a natriuretic effect in these animals. Results suggest that irbesartan treatment ameliorate STZ induced diabetic nephropathic changes, in rats.

KEY WORDS

Irbesartan, Diabetic nephropathy, Streptozotocin.

INTRODUCTION

Diabetic nephropathy (DN) is a common cause of end-stage renal failure (1). The pathophysiology and clinical manifestations of kidney damage however, are duration related. They appear according to a described sequence-beginning with glomerular hyperfilteration and reversible proteinuria and ending in renal insufficiency. Since the pathogenesis of progressive renal damage is multifactorial, the mechanism by which hyperglycemia causes microangiopathy in diabetic glomeruli is still poorly understood. To date, irrespective of the pathogenesis, there is no satisfactory treatment of progressive DN other than attempting to decrease the symptoms. It is controversial whether therapy influences progression of the established DN, however, evidence exists for the effectiveness of control of blood pressure (2), treatment with angiotensin converting enzyme (ACE) inhibitors (3), restriction of dietary protein intake (4), and treatment of dyslipidemia (5). Renin – angiotensin system has also been reported to be an important contributing factor in the pathophysiology of DN. Recently, some studies have demonstrated that irbesartan is renoprotective, independently of its blood pressure lowering effect in hypertensive patients with type 2 diabetes and microalbuminuria (6,7). Keeping in view the possible beneficial effects of angiotensin receptor antagonists in diabetic microalbuminuria, the present study was undertaken to evaluate the role of irbesartan in STZ induced DN, in rats.

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MATERIALS AND METHODS

Adult albino rats (more than six months old) of either sex, weighing nearly 250-300g, were maintained on a standard diet. Food and water were given ad lib.

Induction of Diabetes : Rats were made diabetic with streptozotocin (STZ, sigma USA). A single intravenous injection of STZ was given (50-mg/kg body weight, in 0.05 mol/l of citrate buffer of pH 4.5). Blood sample was obtained from the tail vein after 48 hours of STZ administration and every 4 weeks, thereafter. Animals with blood sugar of more than 250 mg/dl were considered diabetic and used for the study.

Study Outline : Rats were divided into 4 groups of 10 rats each. Group I was used as control and received citrate buffer

only (iv). Group II was given STZ (50 mg/kg, iv.). Animals of group III were given STZ (as in group II) + irbesartan (20 mg/kg, po, daily). To these rats irbesartan was started after 8 weeks of STZ administration and continued for another 8 weeks. Group IV received STZ (as in group II) + regular insulin 4 IU/kg (sc, twice daily). To these rats, insulin was started after 8 weeks of STZ administration and continued for another 8 weeks.

Blood glucose was determined by glucose oxidase-peroxidase method (8). DN was assessed biochemically, by determining urine volume, urinary protein, blood urea, creatinine clearance and urinary electrolytes. For measuring urine volume, each rat was individually housed in a metabolic cage for 6 hours and the volume of urine excreted during this period was measured, accordingly. Urine protein was estimated according to Johnson et al (9). Blood urea was estimated by using diacetyl monoxime (10). Serum and urinary creatinine were estimated by modified Jaffe's reaction (11) and creatinine clearance was calculated. Urinary electrolytes (Na^+ and K^+) were estimated by flame photometry.

Results were statistically analyzed using unpaired Student's 't'-test.

RESULTS AND DISCUSSION

Results of the present study confirm that administration of STZ in rats showed a marked and sustained increase in blood glucose. Irbesartan treatment failed to alter the same, significantly. These results on the effect of irbesartan on blood glucose are in agreement with others who have also shown that irbesartan has no effect on glycemic control in diabetics

Table 1 : Effect of Irbesartan treatment (started after 8 weeks of STZ administration) on urine volume, urine protein, blood urea, creatinine clearance and urinary electrolytes in rats with STZ- induced diabetic nephropathy (values are mean ± SE for 10 animals in each group).

Duration after STZ administration	Control	STZ	STZ + Irbesartan	STZ + Insulin
Urine Volume (ml/6 hours)				
8 Weeks	3.9±0.17	14.2±0.33 *	16.0±0.39 *	15.5±0.52 *
12 Weeks	4.1±0.21	10.3±0.41 *	10.2±0.41 *,**	12.5±0.52 *
16 Weeks	4.3±0.09	6.4±0.55 *,**	6.8±0.28 *,**	9.0±0.42 *,**
Urine Protein (g/dl)				
8 Weeks	Nil	1.6±0.16	1.8±0.10	1.9±0.09
12 Weeks	Nil	1.9±0.16	1.2±0.36 +	1.3±0.12 **,+
16 Weeks	Nil	2.1±0.13	0.9±0.03 **,+	1.0±0.07 **,+
Blood Urea(mg/dl)				
8 Weeks	19.1±0.60	106±3.10 *	110.1±2.88 *	107.2±3.04 *
12 Weeks	21.3±0.86	136.0±7.77 *,**	61.9±1.50 *,**,+	58.2±1.38 *,**,+
16 Weeks	21.6±0.98	150.0±8.43 *,**	41.8±0.85 *,**,+	41.3±0.84 *,**,+
Creatinine Clearance(ml/min)				
8 Weeks	1.1±0.04	0.9±0.12	1.1±0.06	1.0±0.07
12 Weeks	1.1±0.05	0.2±0.11 *,**	1.3±0.11 +	1.6±0.11 *,**,+
16 Weeks	1.2±0.06	0.1±0.03 *,**	1.2±0.07 +	1.6±0.08 *,**,+
Urinary Sodium(meq/l)				
8 Weeks	15.±0.70	14.2±0.33	14.8±0.33	14.5±0.34
12 Weeks	17.1±0.57	13.4±0.37 *	20.3±0.76 **,+	17.4±0.75 **,+
16 Weeks	16.0±0.60	12.8±0.61 *	24.4±0.85 **,+	22.1±0.64 **,+
Urinary Potassium (meq/l)				
8 Weeks	13.5±0.58	12.8±0.44	11.5±0.37	11.4±0.45
12 Weeks	14.9±0.58	12.0±0.45	13.7±0.49 +	12.8±0.44
16 Weeks	14.2±0.56	11.2±0.29	16.4±0.70 **,+	14.5±0.65 **,+

* $p<0.01$ when compared with control, ** $p<0.01$ when compared with STZ, + $p<0.01$ when compared with STZ + Irbesartan

(6). It was further observed in the present study that administration of STZ causes DN since these animals exhibited polyuria, proteinuria, uremia and reduced glomerular filtration rate (GFR). Treatment with irbesartan resulted in a reduction in proteinuria as well as uremia. Irbesartan also improved GFR and increased urinary excretion of electrolytes. Changes in various biochemical parameters except blood glucose were similar to those observed in the group that was given insulin (Table 1). Reduction in urinary protein and blood urea, and increase in electrolyte excretion were directly related to the duration of irbesartan administration. Parving et al (6) showed that antihypertensive treatment with irbesartan has a renoprotective effect in hypertensive patients with type 2 diabetes and microalbuminuria. They also reported that restoration of microalbuminuria to normoalbuminuria, in these patients, was dose dependent. Since STZ exhibited macroalbuminuria, which was also found to be reduced with irbesartan along with the improvement in other biochemical parameters, the results of the present study suggest that irbesartan has a renoprotective effect not only in patients with microalbuminuria but even in higher grades of proteinuria.

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