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RESEARCH ARTICLE

Renoprotection by Telmisartan versus Benazepril in Streptozotocin Induced Diabetic Nephropathy

JANARDHAN SINGH, SALIL BUDHIRAJA, HARBANS LAL, BRIJ RANI ARORA

For author affiliations, see end of text.

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ABSTRACT

Diabetic nephropathy (DN) is one of the major causes of end stage renal disease. Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) are preferred for delaying progression of DN. This study compared the preventive renal effects of telmisartan (10 mg/kg, p.o.), an ARB, that completely blocks angiotensin action, and benazepril (5 mg/kg, p.o.), an ACE inhibitor, which is a partial blocker of angiotensin II production, in an animal model of diabetic nephropathy. DN was induced by streptozotocin (50 mg/kg, i.p.) single injection in male albino rats. Biochemical parameters (creatinine clearance, urinary protein and blood urea) were significantly ($p<0.01$) altered in diabetic rats after 4 weeks. Telmisartan (10 mg/kg, p.o.) and benazepril (5 mg/kg, p.o.) treatment significantly ($p<0.01$) reduced elevated levels of blood urea and urinary protein in diabetic rats. After 8 weeks of diabetes there was significant difference in reduction of blood urea in between telmisartan and benazeril group. Difference between telmisartan and benazepril for reduction in proteinuria was not singnificant. Difference between telmisartan and benazepril for creatinine clearance improvement was also not significant ($p>0.05$). Histology revealed beneficial effects produced by both drugs. This study demonstrates telmisartan is equally renoprotective as benazepril.

Keywords: *Diabetic nephropathy, STZ, Telmisartan, Benazepril*

Diabetic nephropathy (DN) is one of the most serious problems in nephrology, as 40% of the cases of end stage renal disease (ESRD) are due to this entity. Angiotensin II plays an important role in mediating proteinuria by various mechanisms including hyperfiltration, opening of nonselective pores in the ultrafiltration barrier, modifying the composition of glomerular basement membrane and reducing nephrin expression on podocytes. Agents blocking the renin angiotensin system (RAS), angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB), are preferred for preventing albuminuria and delaying progression of DN [1]. Currently, ACE inhibitors are the first line drugs and ARBs are used in those patients who cannot not tolerate ACE inhibitors. There is controversy regarding preferable method for RAS blockade in diabetes, which started after the discovery of ARB. ARBs produce complete blockade of angiotensin II effects while ACE inhibitors partially block the generation of angiotensin II. Crook and Penumalee [2] suggested that there was no significant difference between ACE inhibi-

tors and ARBs with regard to renal outcomes in DN. Abott and Bakris [3] suggested that the greatest benefit for slowing progression of renal disease in DN now belongs to ARB. Hsueh also suggested that effects of ACE inhibitor in slowing the progression to ESRD which have not been clearly demonstrated in type 2 DN [4]. Therefore the present study was undertaken to evaluate the role of an ARB (Telmisartan) and an ACE inhibitor (Benazepril) in streptozotocin (STZ) induced diabetic nephropathy.

MATERIAL AND METHODS

Animal Model

Male albino rats (HAU, Hisar strain) 8-10 weeks old, weighing (230-280 g), were used. Rats were fed with standard food pellets and water *ad libitum*, housed in cages under 12/12 dark/light cycle. The experimental protocol met the national guidelines on the proper care and use of animals in the laboratory research and an

Table 1. Changes in blood glucose levels in each experimental group (values are mean±SEM for 10 animals in each group).

Groups/Drugs	Blood sugar (mg/dL)				
	0 week	4 week	8 week	12 week	16 week
Control	86.7±1.31	88.8±0.89	88.3±1.17	90.8±0.67	89.6±1.18
Diabetic	90.4±0.93	250.7±1.80[*]	252.4±1.66[*]	254.4±1.90[*]	255.8±1.36[*]
Telmisartan	90.6±0.84	243.7±3.07	245.0±2.25	239.0±1.81[**]	243.3±2.43[**]
Benazepril	91.4±0.83	245.2±2.52	244.3±2.46	241.0±2.85[**]	246.1±2.45[**]

[*] $p < 0.001$ when compared with control
[**] $p < 0.05$ when compared with Diabetic

institutional animal ethics committee approved the study.

Study Groups

Following the induction of diabetes, the animals were randomly allocated into four groups (n =10) and treated for 16 weeks as follows:

- GROUP-1: Control (non diabetic) rats.
- GROUP-2: Diabetic rats with no treatment.
- GROUP-3: Diabetic rats treated with the AT1 antagonist, telmisartan (Glenmark Pharmaceutical ltd., Mumbai, India), 10 mg/kg per day by gavage, started 5 days prior to STZ and continued for 16 weeks.
- GROUP-4: Diabetic rats treated with the ACE inhibitor, benazepril (Novartis Pharmaceutical Ltd, Mumbai, India) 5 mg/kg per day by gavage, started 5 days prior to STZ and continued for 16 weeks This was a randomized study with concurrent control and appropriate blinding.

Induction of Diabetes

Diabetes was induced by single injection of streptozotocin (STZ) at a dose of 50 mg/kg, i.p. dissolved in 0.01M citrate buffer (pH 4.5) after 16 h fasting. 72h after STZ injection diabetes was confirmed in rats showing blood sugar level greater than 250 mg/dL. Animals with blood glucose levels greater than 250 mg/dL were considered for further study. Blood samples were collected. 72 hours after STZ administration, and thereafter every 4 weeks from orbital plexus by pricking a needle under ketamine anaesthesia. Glucose-Oxidase assay method [5] was used to determine the blood glucose.

Biochemical Parameters

Nephropathy was evaluated by estimating blood urea [6] and urinary proteins [7]. Further creatinine clearance was also determined as a measure of glomerular filtration rate (GFR). Creatinine clearance was assessed from the urinary [8] and serum creatinine [8] and expressed as mL/min/kg body weight. Blood urea was estimated by diacetyl monooxime method. Serum and urinary creatinine were measured by alkaline picrate method (Jaffe's method). Urinary protein was measured by precipitation with trichloroacetic acid and the precipitate was dissolved in 1N NaOH and quantitated by Biuret method.

Histological Assessment of Kidney Injury

Light microscopy- At 16 weeks, portions of kidney were obtained and fixed in 10% buffered formalin. Staining procedures were performed by standard techniques in our laboratory. [9] Paraffin sections were cut at 3 μm and stained with hematoxylin and eosin, periodic acid-Schiff (PAS) and silver methanamine. All glomeruli in randomly selected sections were examined under high power fields for evidence of sclerosis.

Histometrical measurements- The degree of glomerulosclerosis was evaluated by semiquantitative method [10]. A total of 50 glomeruli from each specimen were graded according to severity of the glomerular damage. The degree of sclerosis in each glomerulus was subjectively graded on a scale of 0 to 4: Grade 0- no change; Grade 1- slight glomerular damage, mesangial matrix and/ or hyalinosis with sclerotic area less than or equal to ¼ of glomerulus; Grade 2- sclerosis of ¼ to ½ total glomerular area; Grade 3- sclerosis of more than ½ the glomerulus but not global and grade 4- global sclerosis.

Mean score of glomerulosclerosis was calculated in each study group and compared.

Statistical Analysis

All data are shown as mean±SEM unless otherwise specified. Results were analyzed by analysis of variance (F-test). Significance between the groups was estimated using Student's t-test. A P value of less than 0.05 was considered as statistically significant.

Table 2. Effect of Telmisartan (10 mg/kg/day, p.o.) and benazepril (5 mg/kg/day, p.o.) pretreatment on blood urea levels in rats with STZ induced diabetic nephropathy (values are mean±SEM for 10 animals in each group)

Group/Drugs	Blood urea level (mg/dL)				
	0 week	4 week	8 week	12 week	16 week
Control	18.4±0.60	18.6±0.60	19.7±0.44	19.3±0.55	20.3±0.39
Diabetic	20.6±0.54	59.0±1.40[*]	60.5±1.34[*]	61.7±0.79[*]	63.8±1.32[*]
Telmisartan	19.6±0.77	27.3±1.03[**]	29.2±1.17[**§]	30.5±1.28[**§]	33.2±1.12[**]
Benazepril	20.0±1.03	31.3±0.82[**]	32.6±1.04[**]	33.8±1.11[**]	35.8±1.37[**]

[*] $p < 0.001$ when compared with control
[**] $p < 0.001$ when compared with Diabetic
[§] $p < 0.05$ when compared with benazepril

Table 4. Effect of Telmisartan (10 mg/kg/day, p.o.) and benazepril (5 mg/kg/day, p.o.) pretreatment on urinary protein excretion levels in rats with STZ induced diabetic nephropathy (values are mean±SEM for 10 animals in each group)

Group/Drugs	Urine Protein (g/L)				
	0 week	4 week	8 week	12 week	16 week
Control	0	0	0	0	0
Diabetic	0	1±0.04[*]	1.5±0.07[*]	1.8±0.04[*]	2.1±0.04[*]
Telmisartan	0	0.2±0.04[**]	0.4±0.06[**]	0.5±0.09[**]	0.5±0.08[**]
Benazepril	0	0.3±0.07[**]	0.5±0.07[**]	0.6±0.07[**]	0.6±0.04[**]

[*] $p<0.001$ when compared with control.
[**] $p<0.001$ when compared with diabetic.

Table 3. Effect of Telmisartan (10 mg/kg/day, p.o.) and benazepril (5 mg/kg/day, p.o.) pretreatment on creatinine clearance in rats with STZ induced diabetic nephropathy (values are mean±SEM for 10 animals in each group)

Group/Drugs	Creatinine clearance (mL/min)				
	0 week	4 week	8 week	12 week	16 week
Control	1.1±0.12	1.1±0.21	1.4±0.23	1.3±0.18	1.1±0.10
Diabetic	1.2±0.12	2.2±0.14[*]	1.0±0.04	0.5±0.03[*]	0.3±0.15[*]
Telmisartan	1.2±0.10	1.4±0.16[**]	1.2±0.07	0.8±0.07[**]	0.5±0.14
Benazepril	1.1±0.13	1.6±0.15	1.0±0.06	0.7±0.04[**]	0.5±0.04

[*] $p<0.001$ when compared with control.
[**] $p<0.001$ when compared with diabetic.

RESULTS

Metabolic parameters. Group 1 (control) animals showed a gradual increase in body weight while group 2 (diabetic control) was associated with marked and gradual reduction in body weight. In group 3 and 4 animals treated with telmisartan and benazepril respectively, the decrease in body weight was suppressed.

In STZ diabetic rats there was a significant ($p<0.001$) and sustained increase in blood glucose levels. Telmisartan and benazepril pretreatment alter blood glucose levels significantly ($p<0.05$) in STZ diabetic rats after 12 weeks. (Table 1).

Biochemical parameters. Creatinine clearance was taken as a parameter to assess GFR. In the early weeks of diabetes, there was a significant ($p<0.05$) increase in creatinine clearance that gradually decreased in later weeks. Group 3 and 4 had decreased rise in GFR in early weeks as compared to diabetic rats but less decline in GFR was observed during later weeks as compared to group 2 rats. However, the beneficial effects of these drugs on GFR were not statistical significant ($p>0.05$) (Fig 1) and the difference between telmisartan and benazepril group was not statistically significant. Urinary protein excretion was not observed in any rat from con-
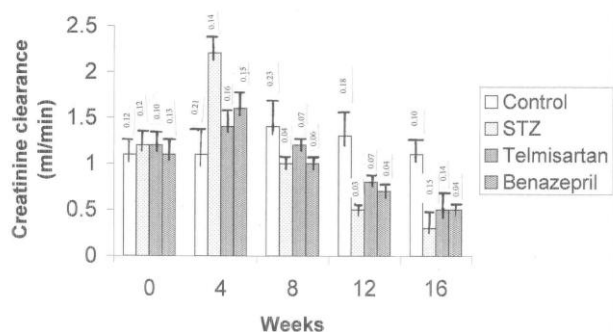
trol group. In the control group there was no excretion of protein in urine. However, there was a significant ($p<0.001$) and sustained increase in urinary protein excretion after 4 weeks of STZ. Telmisartan and benazepril pretreatment significantly ($p<0.001$) retarded the increase in proteinuria compared with the untreated diabetic group (Fig 2). There was no significant difference in reduction of proteinuria between telmisartan and benazepril pretreatment group. There was a significant ($p<0.001$) increase in blood urea in STZ diabetic animals as compared to control group. Telmisartan and benazepril pretreatment significantly ($p<0.001$) reduced the elevated levels of blood urea in diabetic rats (Table 2). However, when compared with benazepril pretreated group, reduction in blood urea level was significantly ($p<0.05$) higher in telmisartan group after 8 weeks.

Histology. Light microscopic findings-Histology of control rat kidney showed normal glomeruli, renal tubules and blood vessels. (Fig 3) After 16 weeks of STZ administration severe and gross changes were observed in kidneys. Diabetic rats had increased glomerulosclerosis and tubulointerstitial injury when compared to control rats. Most of the glomeruli showed diffuse sclerosis while remaining glomeruli also showed segmental sclerosis with basement membrane thickening and increased mesangial matrix expansion with wide spread interstitial

Fig 1. Effect of telmisartan and benazepril pretreatment on creatinine clearance levels.
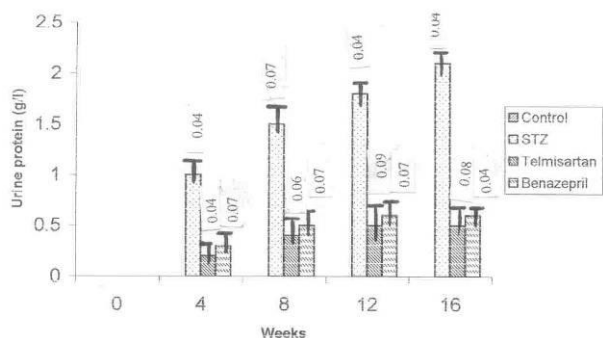

Fig 2. Effect of telmisartan and benazepril pretreatment on urinary protein levels.
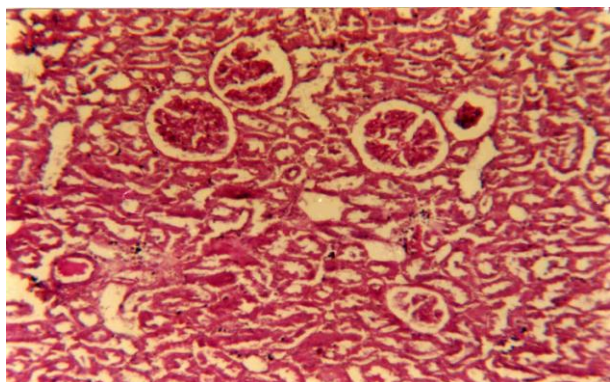
Fig 3. Light micrograph of rat kidney from STZ treated group showing diffuse and global sclerosis of glomeruli. Tubular atrophy and interstitial fibrosis are prominent (original magnification × 100).

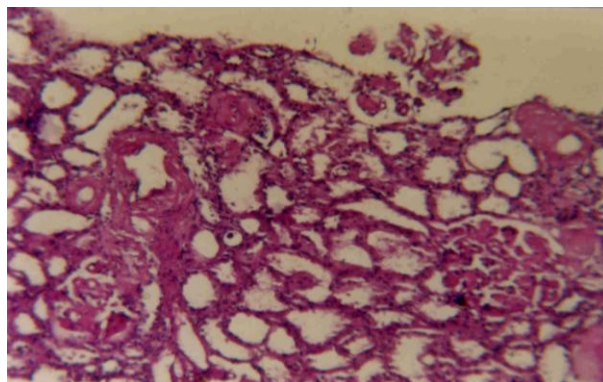
Fig 4. Light micrograph of kidney from rats of benazepril group ing glomeuli with segmental sclerosis, mesangial expansion. Tubular atrophy with interstitial fibrosis is present (original magnification × 100).
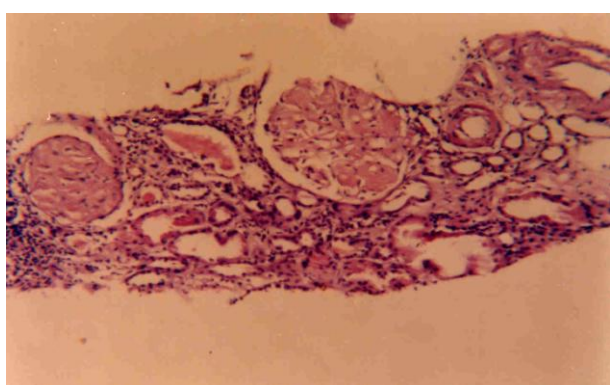

Fig 5. Light micrograph of rat kidney from control group showing normal glomeruli and tubules (Original magnification × 100).
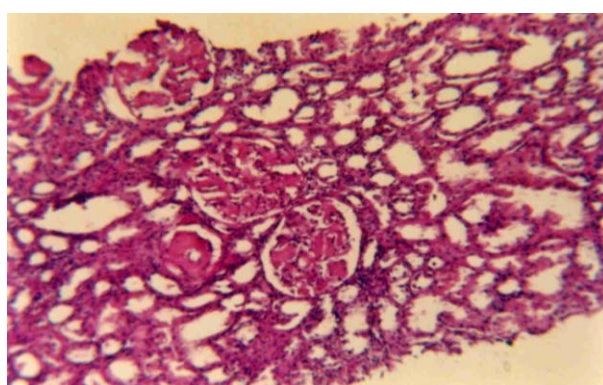

Fig 6. Light micrograph of kidney from rats of telmisartan group showing segmental sclerosis of glomeruli, mesangial expansion. Atrophied tubules and inflammatory cells in the interstitium also seen (original magnification × 100).

fibrosis. Most of the tubules were atrophied (Fig 5). Increased glomerulosclerosis and tubulointerstitial injury in diabetic rats were reduced by telmisartan and benazepril pretreatment. (Fig 4, Fig 6)

Histometrical studies. Mean glomerulosclerosis score was significantly increased in the diabetic rats compared to controls. The score was 3.2±0.42 in diabetic rats. Telmisartan and benazepril prevented the increase in mean glomerulosclerosis score to (1.8±0.42) and (2.0±0.48) respectively.

DISCUSSION

The results of the present study demonstrate that both telmisartan and benazepril attenuate the development of proteinuria, prevent kidney structural injury and elevation of blood urea. This study also shows that telmisartan reduced elevated blood urea level more effectively as compared to benazepril whereas preventive effect on proteinuria and GFR were comparable. These findings suggest that telmisartan may be used as a first line drug in preventing pathological injury in the diabetic kidney.

In diabetic rats glomerular angiotensin II levels are increased due to increase in angiotensinogen [11]. ACE inhibitors do not fully block production of angiotensin II from angiotensinogen because of up regulation of some

non-ACE pathways (chymase enzyme) in kidneys [12]. Angiotensin II is known to produce deleterious effects on kidney by affecting the blood pressure and renal haemodynamics, production of growth promoting and profibrotic factors, renal tubular and glomerular hypertrophy and oxidative stress in kidney [13]. Therefore, blockade of angiotensin II is very important factor for halting the progress of DN. It is well supported by the fact that although ACE inhibitors afford a degree of renal protection, DN continues to progress relentlessly albeit at a slower rate [14].

There is also possibility of increased fibrosis in vascular smooth muscle cells of kidney via modulating TGF-ß, MAPK pathways due to elevated blood levels of bradykinin produced by ACE inhibitors [15]. This may be one of the important factors responsible for decreased effectiveness of ACE inhibitor on long term use.

RAS blockers have preventive action on DN, independent of its decrease in blood pressure effects but ARBs more effectively block biological effects of Ang II. Presently ACE inhibitors are first choice drugs for diabetic nephropathy. It is suggested from our study that ARB are equieffective as ACE inhibitor and can be used as a first line drug to retard the progression of DN and with the use of ARB we can prevent the side effects of

ACE inhibitor. However, more studies are required to confirm it.

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CURRENT AUTHOR ADDRESSES

Janardhan Singh, Associate Professor, Department of Pharmacology, PGIMS, Rohtak-124001 (Haryana-India).

Salil Budhiraja, Senior resident, Department of Pharmacology, PGIMS, Rohtak (Haryana-India) E-mail: salil_budhi@yahoo.com

Harbans Lal, Associate Professor, Department of Pharmacology, PGIMS, Rohtak-124001 (Haryana-India).

Brij Rani Arora, Professor, Department of Pharmacology, PGIMS, Rohtak-124001 (Haryana-India).